ASSESSING THE PREVENTION IMPACT OF HIV COUNSELING AND TESTING IN THE SOUTH AFRICAN CONTEXT

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ABSTRACT

NORA E. ROSENBERG: Assessing the Prevention Impact of HIV Counseling and Testing in the South African Context
(Under the direction of Audrey E. Pettifor)

HIV counseling and testing (HCT) is rapidly being brought to scale in South Africa, yet its impact on sexual behavior and HIV incidence is not well understood. The purpose of Aim 1 was to assess whether HIV-infected persons in HIV-discordant couples were less likely to engage in unprotected sex after HCT. Self-reported behavioral data from 500 HIV-infected South Africans enrolled in Partners in Prevention HSV/HIV Transmission Study was used. The sexual behavior of two groups was compared: HIV-infected persons who had just learned their HIV status from HCT ($\leq$ 7 days before baseline) and those who previously learned their HIV status from HCT ($\geq$ 30 days before baseline). Among those tested $\leq$7 days before baseline the predicted probability of unprotected sex was high at baseline (0.71), declined in month one (0.08) and remained lower at month twelve (0.08). Among those tested >30 days before baseline the predicted probability of unprotected sex was lower at baseline (0.26), declined futher by month one (0.14) and remained lower at month twelve (0.19). These findings suggest HCT lead to substantial reductions in unprotected sex. In the second aim, the effect of HCT on future HIV acquisition among HIV-uninfected youth was assessed. A retrospective cohort study of 3959 HIV-uninfected youth 15-24 years-old was conducted using a demographic and health surveillance from KwaZulu-Natal, South Africa (2006-2011). Young persons who reported knowing their HIV status from HCT were compared to those who reported not knowing their HIV status from HCT for time to HIV seroconversion using marginal structural Cox proportional hazards models. In these models, after weighting for confounding and censoring, HCT was

protective [HR: 0.59, 95% CI: 0.44, 0.78], underscoring the importance of HCT for youth. In the third aim a framework was developed to describe nine "awareness patterns" within HIV-discordant dyads considering both HCT and HIV disclosure together. It was hypothesized that different types of HCT lead to different awareness patterns and that certain patterns are more strongly associated with uptake of and adherence to behavioral and biomedical HIV prevention strategies. Better understandings of these associations may inform how to optimize HCT delivery for prevention.

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AIDS	Acquired Immune Deficiency Syndrome
ACDIS	Africa Centre Demographic Information System
CHCT	Couples HIV Counseling and Testing
CI	Confidence Interval
HCT	HIV Counseling and Testing
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
HR	Hazard Ratio
OR	Odds Ratio
PIP	Partners in Prevention HIV/HSV Transmission Study
PICT	Provider-initiated counseling and testing
RSA	Republic of South Africa
SSA	sub-Saharan Africa
STI	Sexually Transmitted Infection
VCT	Voluntary Counseling and Testing

CHAPTER I: Specific Aims

HIV counseling and testing (HCT), which is rapidly being brought to scale throughout sub-Saharan Africa (SSA), has been promoted as an HIV prevention intervention by UNAIDS and the World Health Organization [1-3]. However, evidence for the prevention effectiveness of HCT in individuals [4] and couples is of limited rigor [5-6]. This deficit makes it difficult to determine how aggressively to promote HCT compared to more rigorously evaluated HIV prevention interventions, and how aggressively to promote individual- versus couple-HCT. Additionally, this question has primarily been studied in adults, and findings may differ in youth ages 15-24.

In SSA, the prevention effectiveness of HCT has been assessed in a number of randomized and observational studies with behavioral endpoints. For those who test HIV-positive, awareness of HIV status has been associated with an increase in protective behaviors [7-16]. For those who test HIV-negative, awareness seems to have neither an overall increase nor decrease in protective behaviors, but considerable variability exists [7, 9, 12, 17-18]. For HIV-discordant couples who test together, mutual awareness has been associated with extremely high levels of behavior change, levels that surpass those of HIV-positive or HIV-negative persons testing individually, [7, 17] though selection factors may play a role [19].

In SSA, persons who test HIV-negative appear slightly more likely to acquire HIV than those who have never tested based on one randomized study [4] and two limited observational studies [12, 18]. However, all had insufficient sample sizes, and did not account for bias due to survey non-participation. Additionally, all were conducted primarily among adults.

In this dissertation, I assessed the relationship between HCT, behavior change, and HIV acquisition as HCT was becoming widely available in South Africa. These issues were addressed in three manuscripts:

In Aim 1 I assessed whether awareness of being HIV-positive was associated with less unprotected sex in HIV-discordant couples. Using self-reported awareness and sexual behavior data collected from three South African sites in the Partners in Prevention HSV/HIV Transmission Study from 2004-2008, I compared those who were tested for HIV recently (in the week before baseline) to those who were tested for HIV previously (prior to the month before baseline) for levels of unprotected sex in the last month at four time points: baseline, month one, month six, and month twelve. *I hypothesized that at baseline those recently tested would report more unprotected sex acts than those previously tested due to lack of awareness for most of the previous month. I also hypothesized that once both groups had been aware of their own HIV status and their partner's HIV status for the entire preceding month that sexual behavior would be comparable.*

In Aim 2 I assessed whether HIV-negative youth who received HCT were more likely to acquire HIV than HIV-negative youth who had not received HCT. In this aim, I used HCT and sero-status data from a large, longitudinal household-based survey collected by the Africa Centre for Health and Population Studies in South Africa. *I hypothesized that HIV acquisition rates would be higher in those who were aware of their HIV status from HCT.* This was the first longitudinal study to assess this question among young persons aged 15-24. Additionally, I expected it would be one of the first studies to assess the relationship between HCT and HIV acquisition with sufficient statistical power and the ability to address time-varying confounding appropriately.

In Aim 3 I developed an Awareness Framework to characterize nine possible combinations of awareness of HIV status within HIV-discordant couples. I also described how this Framework relates to HIV prevention. Awareness of one's own HIV status and one's

partner's HIV status are often considered separately. In this thought piece I developed a framework for considering these two types of awareness together. Existing research was discussed within this framework, research gaps were identified, and strategies to address these gaps were proposed. *This is not a hypothesis-driven aim, but rather a hypothesis-generating aim.*

This dissertation improves upon the existing understanding of awareness of HIV status, sexual behavior, and HIV prevention with a particular focus on the South African context. This understanding is critical for maximizing the prevention impact of HCT.

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CHAPTER II: Background

Significance

Over the last decade, the response to the HIV epidemic in sub-Saharan Africa (SSA) has intensified dramatically. A decade ago, few people had access to HIV treatment and few knew their HIV status. Today, millions of HIV-infected people in SSA have access to life-saving highly active antiretroviral therapy (HAART) [1] and in order to identify HIV infected persons and link them to treatment, millions test for HIV each year [1-4].

HIV counseling and testing in South Africa

In recent years, South Africa, the country with the world's largest burden of HIV [3], has become increasingly aggressive in its response to the HIV epidemic with HIV counseling and testing (HCT) becoming a central pillar of this response. In 2003 HAART became available in the public sector for the first time [5] and HCT was needed to support linkage to care. In South Africa's 2007-2011 HIV/AIDS Strategic Plan, HCT was promoted as a "gateway into HIV prevention, treatment, and care" and aggressive HCT targets were set [6]. However, in 2009 South Africa was not on track to reach these targets, and a national campaign was launched to mobilize many South Africans to get tested from April 2010 to June 2011 [6]. On the supply side, test kits were made available in clinics throughout the country and HCT was encouraged for all persons attending a health facility. On the demand side, there was a mass mobilization campaign with a strong media presence and multi-sectoral involvement [6].

The proportions of South Africans who have ever or recently sought HCT have increased. From 2005 to 2008, the proportion of South Africans who ever sought HCT increased from 31%

to 51%, and the proportion reporting tested in the last year climbed from 13% to 30% [7-8]. Although comparable statistics are not available from 2011, South Africa nearly reached its ambitious target of testing 15 million people during the 2010-2011 campaign [9], suggesting large increases in both the proportions of people ever tested and recently tested.

Different models of HCT have been implemented in South Africa. Voluntary counseling and testing (VCT) is typically client-initiated and can be delivered in a health facility or community based venue [10]. Provider-initiated counseling and testing (PICT) is typically offered to all patients in clinics using an opt-out model, and was introduced in order to increase the volume of people aware of their HIV status. PICT has become common in antenatal settings, resulting in a higher proportion of women than men aware of their HIV status in South Africa and many other sub-Saharan African countries [11-13]. PICT models have also been introduced into other clinical settings, including tuberculosis, sexually transmitted infection, and outpatient [14-16]. There have also been efforts to expand HCT through home-based, mobile, and workplace models in order to reach persons who do not attend clinics regularly [17-18].

In all of these HCT models, there is often a group information session, and almost always an individual pre-test counseling session, the HIV test itself, and an individual post-test counseling session [19]. During the group session, modes of HIV acquisition and transmission are described, as well as HIV behavioral prevention measures. In the individual counseling sessions, these messages are reinforced and discussed in relation to the client's personal risk factors. The HIV test typically consists of a serial rapid testing algorithm with results available on the same day. And in post-test counseling, different messages are given to HIV-infected and HIV-uninfected clients. For those who are HIV-infected, there is discussion of methods for preventing onward sexual transmission of HIV, and for HIV-uninfected persons there are messages about preventing acquisition. Often, a patient commits to a behavioral plan. In VCT models, counseling tends to be longer and more intensive, as counselors tend to have more dedicated time than providers.

HCT is a necessary first step for linkage to care. However, understanding how best to leverage HCT for prevention purposes is less well understood. This dissertation addressed how HCT influences two very different populations in need of HIV prevention interventions: HIV-discordant couples who are primarily in marital and cohabiting relationships and HIV-uninfected youth aged 15-24 living in a high prevalence region in South Africa.

Populations at risk for HIV Acquisition

Aim 1 is conducted in a population of South African HIV-discordant couples. HIV-discordant couples are important from a prevention perspective because the HIV-uninfected partner is at ongoing risk for HIV acquisition through sexual exposure to the HIV-infected partner [20]. In sub-Saharan Africa, there is considerable variation in the share of married and cohabiting couples that are HIV-discordant, with an estimated 1% in Niger and Senegal and 14% in Lesotho [21]. South Africa's epidemic is more similar to Lesotho's, suggesting its share of HIV-discordant couples would be comparable to Lesotho's [3].

There is considerable debate over what share of total transmissions occur within married and cohabiting HIV-discordant couples in generalized epidemics. Estimates range from 14% to 94% of all infections [22-23] with the share of total couples that are married and cohabiting being a key determinant [24]. Even if the fraction of new infections occurring in married and cohabiting relationships is on the lower end of the range, the absolute number of persons acquiring HIV from stable HIV-infected partners is substantial, given 1.8 million annual infections in sub-Saharan Africa [3]. Thus, interventions for these couples are important.

Couples HCT has long been recognized as an important intervention for HIV-discordant couples, and has been implemented in a variety of clinical and community settings in sub-Saharan Africa [25]. Recent guidance from the World Health Organization emphasizes the importance of couples HCT and other couple-oriented strategies [26] and offers suggestions on methods for

delivery. In spite of the numerous benefits and multiple delivery models, the majority of HCT is still conducted among individuals, not couples [26-27].

Aim 2 is conducted in a population of HIV-uninfected youth living in a high prevalence sub-district of KwaZulu-Natal, South Africa. Youth, and especially young girls, are at very high risk for HIV acquisition throughout sub-Saharan Africa [3]. In South Africa, an estimated 21% of girls and 5% of boys acquire HIV by the time they are 24 years old [8, 28-29]. Although there is some indication that incidence rates are declining in South Africa [7], it is unclear if findings reflect a true decline or simply increasing rates of non-participation by HIV-infected youth.

There are a variety of settings where youth 15-24 can get tested for HIV in South Africa. Youth over the age of 12 are able to test for HIV without adult consent [19]. As such, youth can be tested in antenatal and other clinical settings, stand alone HCT services, or home-based or mobile services. Nonetheless, South African youth report lower prevalence of HCT than adults [30].

There are important questions about how best to deliver HCT to youth. Delivering HCT in youth-appropriate ways is critical, as youth often report judgmental care-seeking environments, and this can be a barrier to care [31-32]. Stand-alone youth-only services are one option, but difficult to sustain at a national level. LoveLife, a South African organization dedicated to sexual health among young persons, has proposed a model for improving and then accrediting adolescent-friendly services in existing facilities, rather than creating stand-alone services [33]. This model has been shown to be effective at improving a variety of process indicators [34], but has not been evaluated for uptake of services or health outcomes. Schools are another possible venue for testing of youth. Demonstration projects have found a very high uptake of testing when it is offered in secondary schools, suggesting this may be an effective way of reaching a large volume of youth [35]. In 2011 South Africa announced its intention to introduce HCT into secondary schools [36], but this has not come into fruition due to controversy. There are concerns

about this environment being non-confidential, stigmatizing and deficient with respect to psychosocial support and linkage to care [36-37].

HCT and HIV Incidence

The effect of HCT on HIV prevention is surprisingly not well established. Randomized trials with HIV incidence endpoints are considered the gold standard for assessing the efficacy of HIV prevention interventions [38]. To date, there is only one randomized trial in sub-Saharan Africa assessing the efficacy of HCT with an HIV incidence endpoint [39]. In a cluster randomized trial in Zimbabwean workplaces, participants randomized to intensive on-site HCT were fifty percent more likely to acquire HIV than participants randomized to passive off-site HCT (Table 2.1). Although results were not statistically significant, the trend suggested HCT was not protective.

A second randomized trial, HPTN 043 (Project Accept) randomized communities to either a multilevel HCT intervention (community-based mobile HCT, community mobilization, and posttest support services) or to standard clinic-based HCT [17, 27]. The study, which was conducted in South Africa, Zimbabwe, Tanzania, and Thailand, used community HIV incidence as the primary endpoint. Efficacy data have not been published yet, but early data show that uptake of HCT was much higher in intervention communities [17].

In the absence of randomized trials, non-randomized observational studies with HIV incidence endpoints serve as an important source of evidence. In a large population-based study in Manicaland, Zimbabwe [40] those who received HCT were 30% more likely to acquire HIV than those who did not, although results were not statistically significant. In a large population-based study in Rakai, Uganda [41] results were null and imprecise. In a clinic-based study in Kigali, Rwanda, incidence was slightly higher among HIV-negative women after HCT compared to before, although the effect was very small and not significant [42]. Although none of these studies had sufficient power to detect small effect sizes, all found that awareness of HIV status

was either non-protective with a trend towards elevated rates of HIV acquisition among HIV negative persons (Table 2.1).

Additional observational studies with HIV incidence endpoints have compared those who test for HIV as couples to those who test for HIV as individuals. In Kigali, Rwanda, two years after HIV testing, those who accessed HCT with their partners experienced half the rate of HIV acquisition as those who accessed HCT individually [42-43]. This was the case among HIV-negative women overall [43] and specifically among HIV-negative women in HIV-discordant relationships [42] (Table 2.1). This suggests that couples' HCT may be more effective at reducing HIV incidence than individual HCT.

Studies assessing the relationship between HCT and HIV acquisition have not been conducted among youth, and studies from adults cannot be readily extended to young persons. Understanding how HCT impacts HIV acquisition in this very high risk population is important.

Associations between HCT and Sexual Behavior

Behavioral endpoints are often used as intermediate measures for assessing the effectiveness of behavioral HIV prevention interventions. In assessments of the relationship between HCT and behavior change in SSA, there appears to be a marked contrast between persons who test HIV-negative and those who test HIV-positive [44]. Among those testing HIV-negative, HCT does not appear to have an overall protective or harmful effect, though there is considerable variability between studies [10, 43, 45-47]. However, among those who test HIV-positive, there is a consistent positive association with protective behaviors, especially condom use [44]. This has been seen in longitudinal studies within the same persons over time [45-46, 48-50] and in cross sectional studies comparing aware persons to unaware persons [51-52]. These results are consistent with the results of studies with HIV incidence outcomes.

In addition, several observational studies assessing the effect of HCT on behavior change have been conducted in HIV-discordant couples. In these couples, HCT has a very large effect on

self-reported condom use, an effect that exceeds what would be expected if both the HIV-positive and HIV-negative members of the couple tested separately [10, 42-44, 48, 53]. These effect sizes are consistent with, but more extreme than the results of studies with HIV incidence endpoints. The World Health Organization has recently released guidance emphasizing the importance of couples-based HCT strategies, and suggests several options for achieving these strategies [26].

Although studies of HIV-discordant couples have large effect sizes, their generalizability has been questioned. Recruitment of couples has proven challenging in a number of clinical and research settings [45, 54-55] and those who participate may be more prevention-oriented. It is not known whether the large effect sizes would be seen in the general population.

Conceptual Model

Understanding why HCT may have little effect in HIV-negative persons, but a protective effect in HIV-positive persons and HIV-discordant couples is important. At the individual level the effect of HCT on sexual behavior and ultimately HIV incidence may be mediated by constructs in the "Information-Motivation-Behavioral Skills" (IMB) theoretical framework [56-57]. This framework has been validated extensively in a number of settings, including South Africa, with HIV-positive and HIV-negative persons [57-60]. Within the context of HCT, *information* pertains to basic facts about HIV transmission and prevention, as well as information about one's own and one's partner's HIV status. *Motivation* pertains to one's desires to protect oneself or one's partner from HIV, as well as one's perception of the likelihood of HIV transmission or acquisition. *Behavioral skills* relate to one's perceived ability (self-efficacy) or actual ability to perform risk reduction behaviors. These three IMB constructs can mediate or modify the adoption of a number of sexual behaviors (abstinence, condom use, pre-exposure prophylaxis use, partner reduction, sero-sorting) and care-seeking behaviors (circumcision and HAART for prevention) which could in turn affect rates of HIV transmission or acquisition (Figure 2.1).

HCT likely impacts IMB constructs differentially by HIV status. Learning that one is HIV-positive may increase one's perceived risk of HIV transmission and motivate condom use [61]. On the other hand, learning that one is HIV-negative may lead to misperceptions about personal risk, such as perceived immunity [62], or the belief that one's partner is also HIV-negative. These misperceptions may lead to a decreased motivation to engage in protective behaviors and affirm past risk [63]. Mutual awareness of discordance may lead to the highest level of behavior change because the knowledge of HIV risk within the dyad is shared between both members of the couple as is the motivation to protect the uninfected partner.

The AIDS Risk Reduction model (ARRM) is a second behavioral theory with direct applicability to the relationship between HCT and subsequent sexual risk [64]. ARRM postulates that HIV-related behavior change typically consists of three stages. The first, *labeling*, involves recognizing that one's activities are putting them at risk for acquiring or HIV. The second, *commitment*, entails deciding to alter that risky behavior. The third, *enactment* entails making plans to overcome barriers to risky behaviors (such as communication with a partner) and learning strategies to reduce risky behaviors (such as correct condom use). The counseling portions of HCT sessions map directly onto these three stages.

Additionally, these individual-level characteristics are embedded within a broader social ecology with dyad-level dynamics and larger social structures at play. In particular, the relative importance of these constructs may vary by gender [65-66] or age [67]. In South Africa, a society with wealth and power gaps between men and women [68], gender may be an important moderator of the relationship between HCT and HIV risk. In particular, a woman's motivation to disclose her HIV status and subsequent self-efficacy to negotiate protective behaviors may be dampened by a fear of abandonment, blame, discrimination or violence [69-70]. Men's motivation for risk reduction may be influenced by a fear of exposing unfaithful behaviors [71-73]. Associations between HCT and these constructs may also differ by age. Adolescents are cognitively, emotionally, and behaviorally different from adults [74]. They also have different

types of sexual partnerships. For a variety of reasons, the age of marriage has been rising in South Africa and persons in the 15-24 age range are generally in unions of shorter duration than their older counterparts [75]. For all of these reasons, young persons may experience HCT differently than older persons.

Innovation

There are several key gaps in the understanding of HCT, sexual behavior, and HIV incidence. First, much of the research addressing these topics, especially research with HIV endpoints, was conducted when the care-seeking environment was quite different: HCT was less widely available and ART was largely unavailable. Second, studies conducted among HIV-discordant couples did not account for how quickly after HCT condom use was adopted. Next, studies assessing the relationship between HCT and HIV incidence have not been conducted among young persons, and those conducted among adults had important design limitations [40, 76]. Finally, there are several patterns of HIV status awareness within HIV-discordant couples that have not been well characterized. This dissertation addressed these gaps. I elaborate on them below.

As the HIV care-seeking environment has evolved, the relationship between HCT and sexual behavior must be reassessed. The availability of treatment may alter the perceived severity of HIV. Such a change could lead HIV-negative persons to take fewer precautions to avoid HIV acquisition. Similarly, beliefs that those on HAART are less infectious could lead to behavioral disinhibition among HIV-positive persons and their sex partners. The relationship between HAART and disinhibition has been seen in developed countries [77], though it has not yet been seen in sub-Saharan Africa [78]. In Aims 1 and 2, the relationships between HCT and sexual behavior change were assessed in the era when HAART was being brought to scale. In Aim 1, this relationship was assessed among the HIV-positive index participants in HIV-discordant

couples [79]. In Aim 2, this relationship was assessed among HIV-uninfected youth aged 15-24 in a large demographic surveillance area [80].

The primary innovation of Aim 1 was the assessment of the impact of HCT on behavior change within HIV-discordant couples immediately after HCT. Couples HCT is associated with increased condom uptake [8-12]. However, timing of condom uptake is not well understood, because most studies did not have an assessment in the month after HCT. Thus behavior change could have taken several weeks or months to adopt. It is also not known whether those who continue engaging in unprotected sex engage in fewer unprotected acts. These questions, along with durability of condom use over a one-year period, were addressed in this aim.

The primary innovation in Aim 2 is the assessment of the impact of HCT on HIV acquisition among young persons. Although the relationship between HCT and HIV acquisition has been assessed in adults, it has never been assessed in youth 15-24. HIV-uninfected adults who have received HCT have experienced equal or elevated rates of HIV acquisition compared to adults who have not [40-41]. Elevated infection among testers may be due to behavioral disinhibition among some high risk HIV-uninfected persons [47] or higher risk persons being more likely to seek testing. However, the implications of these findings are unclear for adolescents, who are cognitively, emotionally, and behaviorally different from adults [74]. Understanding how HCT impacts HIV in youth is essential, and may help inform ongoing discussions about whether, when, and how to offer HCT to young persons.

Aim 2 also had novel design strengths that were not used in previous observational research. First, I used marginal structural models (MSMs) to account for time-varying confounders. This is particularly important when the exposure (HCT) in one time period influences confounders (e.g. sexual behavior) in a subsequent time period. In this context, MSMs allow for valid estimation of the exposure and outcome relationship, whereas traditional statistical methods may not [40-41]. Next, I formally correct for selection bias due to refusal. In large population-based sero-surveys HIV-positive persons aware of their HIV positive status are less

likely to participate [81-82]. This has the potential to lead to biased results if persons who were aware of their HIV status were more likely to sero-convert, but less likely to participate at later time points. I used inverse probability of censoring weights to account for this. Finally, none of the previous observational studies had sufficient power to detect the small differences in HIV incidence rates between exposure groups [39-41]. Given the large number of people in the catchment area, extremely high HIV incidence rates [83], and prevalence of HCT, I had sufficient power to detect smaller differences in incidence between HCT groups. Overall, these improvements produced results that were likely more valid and more reliable than previous observational estimates.

In the third aim, a framework for considering several different patterns of HIV awareness was proposed in an Awareness Framework. This novel framework delineates all possible combinations of HCT and HIV status disclosure within HIV-discordant dyads. This is a descriptive aim that discusses how different types of HCT may result in different pattern mixes, and how these different pattern mixes may be associated with uptake of and adherence to behavioral and biomedical prevention.

In summary, this dissertation offers four primary innovations. In Aims 1 and 2, questions about HCT and sexual behavior were revisited in a later time period. In Aim 1 the timing of condom uptake was explored. In Aim 2, the relationship between HCT and HIV acquisition were assessed in a younger population using more rigorous methods. And in Aim 3, a framework for a unified understanding of individual and mutual awareness of HIV status was proposed. Taken together, these three aims have contributed to ongoing discussions about what the prevention benefits of HCT are and how they might be optimized.

Tables and Figures

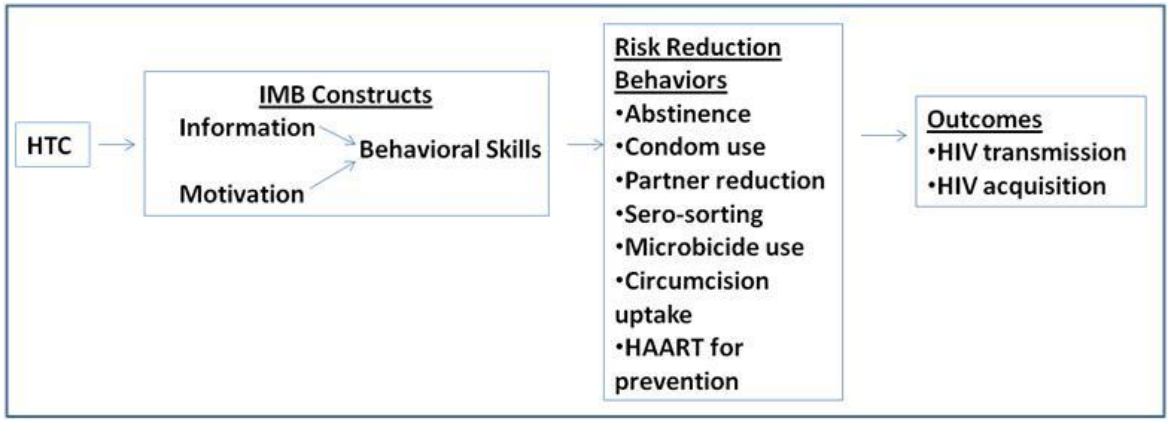
Table 2.1: Studies in sub-Saharan Africa Assessing HCT with HIV Endpoints

Authors, Year	Population/Setting	Design/Exposures	Exposure/Intervention	Results	Conclusions
Testers versus non-testers					
Corbett et al., 2007[39] Harare, Zimbabwe	Predominantly male workers (N=2996) 2001-2004	Cluster randomized trial in 22 workplaces. HIV status ascertained at BL and at 2 years.	Intensive: on-site rapid VCT (71% uptake) Standard: voucher for community-based VCT (5% uptake)	Intensive: 1.37/100 PYs; Standard: 0.95/100 PYs Incidence rate ratio: **1.5 (0.8, 2.8)**	The group that tested had higher HIV acquisition rates, but not statistically significant
Sherr et al., 2006[40] Manicaland, Zimbabwe	Adults in the general population (N=~10,000 PYs) 1998-2003	Prospective population-based cohort study HIV status ascertained from 1998-2000 and then from 2001-2003	*Males* Tested and given results (12%) Not tested (74%) *Females* Tested and given results (10%) Not tested (86%) *Results not reported for those tested who did not receive results	Male w/ results: 2.2/100 PYs Male non-testers: 2.1/100 PYs Incidence rate ratio: **1.1(0.6, 3.8)** Females w/ results: 2.4/100 PYs Female non-testers: 1.6/100 PYs Incidence rate ratio: **1.6 (0.6, 3.8)** Overall w/ results: 2.3/100 PYs Overall w/o results: 1.8/100 PYs Incidence rate ratio: **1.3 (0.8, 2.1)**	The group that got tested had higher HIV acquisition rates, but not statistically significant
Matovu et al., 2005[41] Rakai, Uganda	Adults in the general population (N=3158 people, N=2631 PYs) 1999-2000	Prospective population-based cohort study	*Males* VCT acceptors (62%) VCT non-acceptors (38%) *Females* VCT acceptors (62%) VCT-non-acceptors (38%)	Male acceptors: 1.5/100 PYs Male non-acceptors: 1.1/100PYs Incidence Rate Ratio: **~1.4** Female acceptors: 1.6/100 PYs Female non-acceptors: 1.7/100PYs Incidence Rate Ratio: ~**0.9** Overall acceptors: 1.6/100 PYs Overall non-acceptors: 1.4/100PYs Incidence Rate Ratio: ~**1.1 (p=0.6)**	The group that accepted testing had higher HIV acquisition rates, but not statistically significant

Matovu et al., 2007[75] Rakai, Uganda	Adults in the general population in 2 or more annual surveys N=6637	Prospective population-based cohort study	*Overall* VCT repeat acceptors: 24% VCT 1st acceptors: 40% VCT-non-acceptors: 36%	*Overall* VCT repeat acceptors:1.4/100 PYs VCT 1st acceptors: 1.6/100 PYs VCT-non-acceptors: 1.6/100 PYs Incidence Rate Ratio: **1.0**	Repeat acceptors seemed slightly protected compared to first time-acceptors or non-acceptors. Results were not significant.
Individual testers versus couple testers					
Allen et al., 1992[42] Kigali Rwanda	N=945 HIV-negative women Followed from Pre:1986-1988 Post: 1988-1990	Prospective cohort study with a pre-post and exposed/ unexposed groups	*Females* Tested individually: 667 Tested with partner: 278	Individually aware Pre:2.8/100 PYs, Post:2.9/100 PYs Mutually aware Pre:4.2/100PYs, Post:1.4/100PYs ate ratio (post): **0.5**	The group that tested in a couple had a significantly lower acquisition rate.
Allen et al., 1992[43] Kigali, Rwanda	Women seeking ANC who tested HIV-negative individually or with HIV-positive male partner followed for 2.2 years.	Prospective cohort study with modeling component. Proportion of HIV-negative women testing individually who were in HIV-discordant couples was modeled.	*Females* Tested individually: 1160 PYs Tested with partner: ~60 PYs	Individually aware probable discordant couples: 22/100 PYs Mutually aware discordant couples: 9/100 PYs Incidence Rate Ratio: **0.4**	The group that tested individually had presumably higher HIV acquisition rates. Results were not significant.

Caption: Table 2.1 summarizes completed studies in sub-Saharan Africa of HCT with HIV endpoints.

Figure 2.1: Information, Motivation, Behavioral Skills Theoretical Framework



Caption: Figure 2.1 depicts how the Information-Motivation-Behavioral Change theory relates HIV counseling and testing to HIV prevention.

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CHAPTER III: Resarch Design and Methods

The study designs, study populations, exposure and outcome assessments, and statistical methods for aims 1 and 2 were distinct. In this chapter, I describe the entire approach for Aim 1 before proceeding to the approach for Aim 2. Since the third aim is not data-driven, this approach is described only briefly.

Aim 1

Study Design:

In the first Aim I assessed whether awareness of being HIV-positive was associated with less unprotected sex in HIV-discordant couples. This aim relied on behavioral data from the Partners in Prevention HSV/ HIV Transmission Study (PIP) conducted among HIV-discordant couples in sub-Saharan Africa. PIP was a Phase III randomized placebo controlled trial. It was designed to assess the effect of acyclovir taken daily by HIV-1/HSV-2 co-infected "index participants" on disease progression [1] and HIV-1 transmission to HIV-uninfected sex "partners" [2]. HIV-infected index participants in both the intervention and placebo groups were seen monthly to receive intervention or placebo tablets, condoms, and counseling. In addition, at monthly visits, biomarkers were collected and index participants were assessed for sexual behavior in the previous month.

The underlying research question in Aim 1 is whether HIV-positive persons who know their HIV status are less likely to have unprotected sex than HIV-positive persons who do not know their HIV status. In observational analysis, this is typically assessed through comparisons of sexual behavior in the same persons before and after learning their HIV status, or through

comparisons of sexual behavior in persons who do and do not know their HIV status. Because everyone in PIP was aware of their HIV status at baseline, neither of these comparisons is possible. However, many participants had learned their HIV status in the month preceding baseline (≤30 days), and some of these participants had even learned their HIV status in the week preceding baseline (≤7 days). Therefore some participants were aware of their HIV status for only a small part of the previous month (≤7 days) and others had been aware of their HIV status for the entire previous month (>30 days). For participants aware for the entire previous month, all reported sexual activity must have occurred *after* learning their HIV status. For participants recently aware, some sexual activity may have occurred *before* learning their HIV status. This baseline comparison was used to approximate the comparison between persons who do and do not know their HIV status. Comparisons of self-reported sexual behavior at baseline with self-reported behavior afterwards approximate the comparison of the same persons before and after learning their HIV status.

The primary analysis compared those who learned their HIV status ≤7 days before baseline to those who learned their HIV status >30 days before baseline. I assessed whether the recently aware group was more likely to report unprotected sex and a higher relative number of unprotected sex acts in the last month at baseline, month one, month six, and month twelve (Figure 3.1). One strength of this analysis was that it allowed for comparisons between groups at each point in time, as well as a comparison within each group over time. The baseline comparison was included to compare those aware for the full month to those aware for part of the month. The month-one comparisons were included to compare whether behavior change in both groups occurred immediately. The six and twelve month comparisons were used to assess whether behavior change in each group was maintained over time.

At the baseline cross section, I also conducted a sensitivity analysis on the time since HCT cut-point. In addition to comparing sexual behavior among persons tested >30 days before baseline to persons tested ≤7 days before baseline, I also compared sexual behavior among

persons tested >30 days before baseline to persons tested 8-14 days before baseline and persons tested 21-30 days before baseline. These additional comparisons allowed for assessments of whether there was a dose-response relationship between the amount of time someone was unaware of their HIV status and both the odds of unprotected sex and relative number of unprotected sex. If awareness was protective, persons aware for less time (e.g. 7 days) would be more likely to report any unprotected sex and more likely to report more unprotected sex acts than those aware for more time (i.e. 8-14 or 15-30 days) (Figure 3.2).

This study addressed a question that was similar to questions from previous behavioral research among HIV-discordant couples [3-7]. However, previous studies were conducted prior to the scale-up of treatment, whereas PIP was conducted from 2004-2008, when HAART was being brought to scale. This context could have altered the motivation to change behavior, if availability of treatment altered the perceived severity of HIV acquisition. In addition, this was the first study to be conducted in South Africa, which is often characterized by union instability [8]. Findings may have differed in this social context. Findings from this study were qualitatively compared to the findings of previous work conducted at early time periods in other parts of SSA.

Study Population

The entire PIP trial enrolled 3408 couples from 14 sites in seven sub-Saharan African countries. This analysis relied on data from 508 HIV-infected index participants enrolled from 2004-2007 at the three South African sites: Gugulethu, Orange Farm, and Soweto. Each couple was followed for 12-24 months between November 2004 and October 2008.

All three sites are situated in populous areas with predominantly black inhabitants. Gugulethu is a township located 10 miles outside of Cape Town with a population of 340,000. Orange Farm is a township located 30 miles outside of Johannesburg with a population of 500,000. Soweto is a lower middle class urban part of the city of Johannesburg with a population of 1.2 million. The adult HIV prevalence in all three sites is high: 27% in Gugulethu and 30% in

both Orange Farm and Soweto [9]. At all three sites, ARV rollout began prior to the beginning of the study period [10-12].

Recruitment

Recruitment and initial screening for the study were conducted using individual and couples HCT in both HIV testing and HIV clinical facilities at all three sites. In the larger study 51,900 couples were screened. The prevalence of HIV-discordance at the three South African sites was 31% at Gugulethu, 28% at Orange Farm, and 23% at Soweto [9].

Eligibility

Of those who were screened and identified as HIV-discordant, 48% (N=3135) did not meet study eligibility criteria or decided not to enroll. The eligibility criteria are listed below, along with the proportion of couples ineligible for each reason.

- The HIV-positive index participant had a CD4 count $\geq$250 (54%)
- The HIV-positive index participant had an AIDS-defining illness (2%)
- The HIV-positive index participant was HSV-2 sero-positive (14%)
- The couple had been sexually inactive for the previous three months or likely to terminate the relationship in the next 24 months (5%)

In addition, 20% of couples did not participate for other reasons.[13]

At the three South African sites, three quarters of index participants were female, a fraction that reflects the higher prevalence of HIV among women in South Africa and the higher rates of testing among women [14-15]. The mean age was 33 years (SD=8.5) and the mean education level was 9.4 years (SD=2.8). The majority of indexes (82%) had at least one child. Few (<5%) reported more than one sex partner in the last month. One third of couples were neither married nor cohabitating, half were not married but were cohabitating, and 16% were both

married and cohabitating. The average relationship length was 4.4 years (SD=5.1). On average, males were 4.1 (SD=6.4) years older than females, regardless of which partner was HIV-positive. Few indexes reported relationship violence in the last month.

A small minority of South African index participants (<1%) enrolled with two HIV-uninfected partners. For this analysis, only the sexual behavior pertaining to the first enrolled partner was analyzed. If a second partner was enrolled, this person was categorized as an additional sexual partner, along with other additional reported partners.

External Validity

The recruitment procedures and eligibility criteria lead to several questions about the generalizability of the study population. First, persons who presented for testing as couples may have differed from persons who presented individually or did not present at all. They reflect a subset of the population in which both members of the partnership were willing to learn their own results and disclose their results to one another. In a society that has been characterized by male-dominated decision-making and high rates of partner violence [16-18], this may reflect a subset of couples where power is shared more equitably. The very low baseline rates of reported partner violence suggested that this may be the case [19]. They may also reflect couples in which one or both members had previous consecutive or concurrent partnerships but not current concurrent partnerships at the time of enrollment. In addition, couples in the study population may have been more stable than couples in the general population. The fact that all were willing to enroll for a two-year period and almost all were married or cohabitating supports this notion as there have been documented declines in marriage and cohabitation in the South African social structure [20]. Finally, because of the CD4 and disease progression eligibility criteria, these couples represented persons at an earlier stage of HIV natural history.

Exposure Assessment

The primary exposure for aim 1 is time since diagnosis dichotomized as those who learned their HIV status ≤7 before baseline versus those tested >30 days before baseline. Two other categories were also explored: persons tested 8-14 and 15-30 days before baseline. At baseline, the three groups that reported ≤30 days could have had unprotected sex before they were aware of their HIV status. Those diagnosed >30 days before baseline could have only unprotected sex only after they were aware of their HIV status. Among all of the indexes, the median time since diagnosis was 29 days (IQR: 11 days, 9.2 months).

Outcome Assessment

Unprotected sex was characterized based on index self-report at baseline and then every month thereafter. In this analysis, data is used from four of those time points: baseline and months one, six, and twelve. At each time point, indexes were first asked how many times they had sex with their study partner in the last month. If indexes reported at least one act, they were then asked how many times a condom was used. From these questions, the primary outcome was determined: a) whether or not the index had any unprotected sex in the past month and b) the number of unprotected sex acts in the past month. These two behavioral endpoints were selected because they determined whether and how often a partner was exposed to HIV, the measures of greatest public health relevance. In addition to these primary outcomes, I calculated whether or not participants had any sex acts at all with their study partner in the past month.

For previously diagnosed indexes (>30 days before baseline), all unprotected sex acts reported at baseline must have occurred *after* becoming aware of their HIV status. For recently diagnosed indexes (<7, 8-14, and 15-30 days before baseline) unprotected sex occurred *either before or soon after* becoming aware of their HIV status. At the one, six, and twelve-month time periods, all unprotected sex acts in both groups occurred *after* diagnosis.

Information Bias

Although the larger trial was prospective, this particular analysis ascertained information about the exposure and the outcome retrospectively. It is possible that this would lead to differential misclassification [21]. At enrollment, those who knew their HIV status for the entire previous month may have been less likely to report any unprotected sex in that last month (i.e. higher social desirability). This could have biased results away from the null.

Statistical Analyses

The first set of analyses was longitudinal. I compared recently and previously diagnosed indexes at four time points: baseline, month one, month six, and month twelve. Generalized estimating equations with robust variance estimators were used to account for within-subject clustering at the three different time points. Robust variance estimates accounted for within cluster correlation. An exchangeable correlation matrix was specified for within-subject correlation. An exchangeable correlation matrix assumes that correlations within the same subject, but between different time periods, are equal. This assumption is justifiable in settings where variation between subjects is expected to be stronger than variation within subjects, an assumption that is typically justifiable in repeated measures studies [22].

Two types of models corresponding to the two main outcomes (binary and continuous) were used. The first compared the *odds* of unprotected sex in the last month at each time point. For this outcome, I used a generalized linear model with a logit link and a binomial distribution. This specification of a generalized linear model provided an estimate of the odds ratio at each cross section. The second model compared the *relative number* of unprotected sex acts in the last month at each time point. To compare the relative number of acts between the two groups, I used a generalized linear model with a log link and a negative binomial distribution. This specification of a generalized linear model provided a direct estimate of the relative number of events at each cross section.

Informal exploratory assessments of the data suggested that the frequency of unprotected sex was over-dispersed (i.e. the conditional mean was smaller than the conditional variance), suggesting a negative binomial model would be the better fit for the data. Since Poisson models are nested within negative binomial models, a likelihood ratio test (LRT) comparing the negative binomial model to the Poisson model was used to compare this relationship formally. In this setting, an LRT compares the hypothesis that the dispersion term in the negative binomial model leads to no improvement in model fit over the Poisson model. Rejecting the null hypothesis implies the dispersion term is necessary and the data are over-dispersed.

In both unadjusted models, I included a variable for the main exposure (aware), an indicator for month one, six and twelve time periods, and interaction terms between the main exposure and each of the two later time periods. These equations can be expressed as follows:

[1] $\text{Ln}[\text{Pr}(Y)] = \alpha + \beta_1 X_{\text{aware}} + \beta_2 X_{\text{time1}} + \beta_3 X_{\text{time6}} + \beta_4 X_{\text{time12}} + \beta_5 X_{\text{aware*time1}} + \beta_6 X_{\text{aware*time6}} + \beta_7 X_{\text{aware*time12}}$

[2] $\text{Ln}[\text{Count}(Y)] = = \alpha + \beta_1 X_{\text{aware}} + \beta_2 X_{\text{time1}} + \beta_3 X_{\text{time6}} + \beta_4 X_{\text{time12}} + \beta_5 X_{\text{aware*time1}} + \beta_6 X_{\text{aware*time6}} + \beta_7 X_{\text{aware*time12}} + \gamma_{\text{over-dispersion}}$

I also conducted a baseline cross sectional analysis to assess whether those more recently diagnosed were more likely to report any unprotected sex at baseline. Preliminary inspection of the data suggested that zero-inflated models would be needed for the baseline data to account for the high proportion of participants reporting zero unprotected sex acts in the last month. I first compared Poisson and negative binomial (NB) regression models using a likelihood ratio test. I then compared the better of these two models to a Zero-inflated model using a Vuong test for non-nested models[23]. Graphical comparisons of the predicted probabilities were also used to assess which model was the best fit for the data.

Zero-inflated models are used when it is expected that data are generated from two separate processes: one that generates only zeroes (i.e. no unprotected sex), and another that generates a count (i.e. some number of unprotected sex acts). Zero-inflated NB and Poisson models provided a parsimonious and powerful way of modeling both processes at once. By solving two simultaneous equations, these models generated two sets of parameter estimates. The first set was generated using a logistic regression procedure. This set was used to compare the odds of zero unprotected sex acts between the two exposure groups. The second set of parameters was generated using a NB regression procedure. This procedure was used to compare the relative number of unprotected sex acts between the two exposure groups.

In both the longitudinal and cross sectional models direct acyclic graphs (DAGs) were used to identify individual and couple characteristics that could confound the association between time since diagnosis and unprotected sex [24]. The individual-level variables were gender, age, education level, parity, and having had more than one sex partner in the previous month. The couple-level variables were marital and cohabitation status, relationship length, relationship violence in the last three months, and male-female age difference. All of these variables have been shown to be associated with HIV testing, sexual behavior, or both, in multiple studies in sub-Saharan Africa.

For both the longitudinal and cross-sectional models, interaction between the main exposure and key covariates of public health relevance (gender, age, and site) were assessed prior to confounding. Maximum likelihood methods were used to assess whether an interaction term led to a significantly greater model fit at an alpha level of 0.1.

All variables that were not considered interaction terms were then assessed for confounding. To determine which variables to include in the final multivariate model a backward elimination change-in-estimate process was used. I started with all covariates described above and assessed the resulting change in estimate of the main exposure. Covariates were retained if estimates on the log scale changed by more than 10%. Covariates least likely to result in

confounding were removed first. This order was determined by bi-variable exploratory analyses and subject matter knowledge. All analyses were conducted in SAS v.9.2. (SAS Institute, Cary, North Carolina).

Aim 2

Study Design

In the second aim, I assessed whether HIV-negative youth who received HCT were more likely to acquire HIV than HIV-negative youth who did not receive HCT. For this aim I used data from a demographic surveillance system collected by The Africa Centre for Health and Population Studies in KwaZulu-Natal, South Africa. When the Africa Centre Demographic Information System (ACDIS) was initiated in 2000, primarily demographic, social, and health indicators were collected [25]. Starting in 2003, a sero-survey, that included HIV status, was collected on an annual basis. These annual assessments have continued until the present.

One challenge for studying the relationship between HCT and HIV acquisition is that the exposure and the outcome both involve HIV testing. For meaningful analysis, it is essential that all participants have been tested for HIV (so outcome information is available), even though some participants do not know their own HIV test results (so different exposure statuses can be compared). In this analysis, the exposure and outcome were ascertained through separate mechanisms. The primary exposure (whether or not someone sought HCT outside of the survey) was be ascertained by behavioral self-report. The primary outcome (HIV acquisition), was ascertained from blood provided for the ACDIS sero-survey. ACDIS participants who provided blood for the sero-survey did not learn their HIV status through survey participation during the study period. Rather, participants were encouraged to seek free HCT in the community to learn their status.

The current analysis built upon the strengths of ACDIS and the sero-survey. First the sero-survey was longitudinal, allowing for the temporal observation of HIV testing and HIV

acquisition. Second, the cohort was large and the HIV incidence rate was one of the highest in the world [26-27]. HIV incidence rates allowed for a sufficient sample size to detect even small differences between exposure groups. Third, the methods for ascertaining HCT (self-report) were distinct from the methods for ascertaining HIV status within the survey (laboratory-based antibody assays without return of results). These distinct methods for ascertaining exposure and outcome information were essential for this analysis as HIV status was available even for persons who had never received HCT. Finally, the dataset had a rich set of covariates collected at multiple time points which allowed for control of time-varying confounding [28].

Study Population

ACDIS contains information from a demographic surveillance area located in the Umkanyakude district in northern KwaZulu-Natal, South Africa (Figure 3) [25]. The area is 435 square kilometers and predominantly rural, though it contains an urban township and informal peri-urban settlements [28]. The area houses approximately 86,000 residents and non-residents [25] who are primarily Zulu-speaking [28].

Nested within ACDIS is the population-based HIV sero-survey that contains information about participants' knowledge, attitudes, and sexual behaviors; care seeking behaviors; and knowledge and beliefs about antiretroviral therapy. In addition, everyone was asked to provide a blood sample for laboratory-based HIV testing. Before 2007, there was an age eligibility requirement of 15-49 for women and 15-54 for men. However, starting in 2007, all persons over 15 were eligible to participate [25].

The catchment area is hard-hit by the HIV epidemic. The 2004 adult HIV prevalence was estimated to be 13.5% in adult men and 26.8% in adult women [27], although a reanalysis of these data suggest that these are underestimates [29]. The rate of new infections also remains high with a relatively constant annual HIV incidence of 3.4 per 100 person-years (95% CI: 3.1, 3.7) between 2003 and 2007, although this rate differs considerably by age and gender [26]. Assuming

constant prevalence and the absence of a competing risk of death, the cumulative risk of infection is 74% for women by age 49 and 78% for men by age 54 [30].

HCT in the Subdistrict

The community has had access to several different forms of HCT outside of the ACDIS sero-survey. Since 2000, HCT was available in the district hospital. In order to support decentralized rollout of ART in the catchment area, HCT was introduced into all of the primary health centers beginning in 2004-2005 [31]. Counselors provided HCT service both to clients who presented specifically for HCT and to patients who were referred from other clinical services (tuberculosis, STI, and ANC) [32]. During the national HCT campaign from 2010-2011, the nature of these services did not change, but the volume of services increased. HCT was offered to all persons over 12 presenting to the primary health clinics. Although provider referral is quite common, provider-initiated testing is rare. Almost all testing is performed by counselors.

There are other forms of HCT in the catchment area, as well. Private outpatient clinics, and the district hospital also offer HCT. In addition a local NGO offers HCT in some secondary schools and the President's Emergency Plan for AIDS Relief has supported the Africa Centre to implement home-based HCT and community-based HCT in remote parts of the catchment area starting in 2008-2009 [33].

Starting in 2010, there was a question on the sero-survey about where persons had sought HCT. Of 2771 youth who responded to this question in 2010, 54% reported that it was in a primary health clinic, 20% reported that it was through home-based testing, 7% reported mobile testing, 3% reported a private clinic, 3% reported in the hospital, and 13% reported other. Many of the "other" responses were the specific names clinics or physicians in the catchment area.

There has been a steady increase in HCT over time among youth in the catchment area. The proportion of youth 15-24 who were aware of their HIV status from HCT increased from 27% in 2007 to 52% in 2009 to 59% in 2011 [34]. In 2011, females were 1.9 (95% CI: 1.8, 2.1)

times as likely as males to be aware of their HIV status and youth were 7.2% (95% CI: 6.2%, 8.2%) more likely to be aware of their HIV status with each one year increase in age ($\leq$16 years=41%, 24 years: 76%). Persons who were HIV-infected were 1.3 (95% CI: 1.2, 1.4) times as likely to be aware of their HIV status as HIV-uninfected persons, but this effect was negligible after adjustment for age and gender (prevalence ratio: 1.0, 95% CI 0.9, 1.0).

Eligibility for the Current Analysis

To be included in this analysis, persons must have met the following eligibility criteria:

- Was a resident in the ACDIS demographic surveillance area or a randomly selected non-resident who participated in ACDIS.
- Participated in the sero-survey at least two times between 2006 and 2010.
- Reported whether or not they had received HCT in at least two sero-survey rounds. The first report had to be no HCT, the second report could be either HCT or no HCT.
- Tested HIV-negative in at least one sero-survey before the baseline time point and then tested again (either HIV-negative or HIV-positive) in at least one subsequent sero-survey round after the baseline time point.
- Was 15-24 years old at the time of their first report of HCT.

Recruitment

Fieldworkers make rigorous attempts to contact all persons in the catchment area. For each survey round, teams of two trained fieldworkers visit eligible persons in their households. If they are not present at the first attempt, they are re-contacted up to three additional times. For subjects who moved from one time period to another, up to ten attempts are made to find the individual at the new place of residence, including outside the catchment area [28].

Participation in the Risk Set

When conducting time-to-event analysis, the choice of a time scale is important, as the model implicitly adjusts for time on that time scale [35-36]. Regardless of which time scale is selected, the total person time and the total number of events are the same. However, the risk sets at each event time are different. This can lead to different persons being compared to one another. A graphical depiction of this is presented in Figure 3.2.

The primary time scale that I used in this analysis was time since HCT. This was selected as the primary time scale since because it relates directly to the research question. I also explored time on study as a sensitivity analysis [36]. Other time scales, including age, time since 2006, and time since sexual debut were controlled for analytically.

Given the dynamic nature of the cohort there was the potential for late entries (i.e. people entering the study after their origin) on most time scales. By requiring that persons report *not* receiving HCT at the first time point, it was possible to estimate the time when they did get HCT. Persons were at risk for HIV acquisition from the time they entered the risk set until the time they acquired HIV or were administratively censored due to reaching the end of the follow-up period (2011), refusal, or leaving the cohort through death, out-migration or incarceration.

Internal Validity

The number of eligible residents was approximately 25,000 in the first three rounds of survey collection. Of those eligible, the proportion contacted was 77% in 2003/2004, 98% in 2005, and 92% in 2006. And of those contacted, the proportion who consented to testing within the ACDIS sero-survey was 58% in 2003/2004, 41% in 2005, and 38% in 2006. (Exact numbers are not published from 2007-2010, though 2006 numbers can be used as a proxy).[1]

The high proportion of missing data from the ACDIS sero-survey raises concerns about validity due to selection bias. Selection bias takes the structural form shown in Figure 3.3 [37],

[1] This is based on personal communication with investigators.

conditioning on a common effect. In this example, HCT is the exposure, observed HIV acquisition in the ACDIS sero-survey is the outcome, and participation in the ACDIS sero-survey is a form of conditioning (i.e. restriction).

Those who sought HCT and learned they were HIV-positive may have been less inclined to participate in the sero-survey than those who sought HCT and learned they were HIV-negative or those who never sought HCT. These associations have been seen in sero-surveys in other populations and lead to underestimates of HIV prevalence [38]. However, our study looked at HIV incidence.

Exposure Assessment

In Aim 2, the primary exposure was whether or not someone received HCT and learned their HIV results outside of the ACDIS sero-survey. This exposure was based on behavioral self-report collected in the ACDIS sero-survey from 2006-2010. Table 3.1 displays the items included in the individual questionnaire, the response options, and the periods when each question was asked. For this analysis the following question was used: "Do you know your HIV status from previous testing?"

Because the response to this question may change over time, this exposure is time-varying and each participant could contribute person-time to either or both exposure groups. All participants who report HCT at the time of their first visit were considered "exposed" for the entire follow-up period. All participants who did not report HCT during any of their follow-up periods were considered "unexposed." All participants who reported HCT at some point during follow-up have their person-time split between two exposure groups. The primary exposure was estimated based on the timing of the *first* HIV test in the following way:

- For participants who reported not knowing their HIV status from previous testing in their first survey round and knowing their HIV status from previous testing in the second

survey round, the estimated date of first test was calculated as the midpoint between these two survey dates.

- For participants who reported knowing their HIV status "from previous testing" at a subsequent survey round, the date of first test was calculated as the midpoint between the date of the previous survey round and the subsequent survey round. The person-time contributed before the date of the first test was considered "unexposed" and the time after was considered "exposed."
- Participants who never reported having HCT were considered "unexposed" for the entire study duration. Their time started at the midpoint between their first two reports of not knowing their HIV status.

In this analysis, there was potential for exposure misclassification resulting from behavioral self-report. In a validation sub-study of HIV awareness in the Kenya AIDS Indicator Survey, 44% of HIV-positive respondents reported knowing their HIV status. However, of these, the majority believed that they were HIV-negative [39]. This could have been attributed to receiving an HIV-negative test result prior to HIV sero-conversion. However, it could also stem from denial, social desirability, recall error, misunderstanding of the question, or misperceived HIV status. The possibility of information bias was a concern in this analysis, as well, although in our case the question about HCT exposure was asked among persons when they were HIV-uninfected.

Outcome Assessment

In Aim 2, the primary outcome was HIV acquisition. HIV acquisition was determined by one negative HIV test result followed by one subsequent positive HIV test result. The timing of the HIV acquisition event was calculated as the midpoint between the date when the last HIV test

was negative and the first HIV test was positive, minus 30 days. The reason for subtracting 30 days is to allow for a window period between the time HIV was acquired and detected [40].

For consenting participants, blood was obtained via finger prick and prepared into dried blood spots. A serial HIV testing algorithm with two ELISAs is used: Vironostika HIV-1/HIV-2 Microelisa System (Biomérieux, Durham, NC, USA), followed by Wellcozyme HIV 1+2 GACELISA (Murex Diagnostics, Benelux B.V., Breukelen, The Netherlands) [25].

Temporality of Exposure and Outcome

Because the dataset was interval censored, some persons reported HCT and acquired HIV during the same survey round. When this occurred, the change in outcome always preceded the change in exposure. This is a function of the exposure being classified as the midpoint between two HIV tests and the outcome being classified as the midpoint between two HIV tests, minus one month. This classification helped to avoid a reverse causality problem. A sensitivity analysis was conducted to determine the degree to which this might be biasing the results.

Statistical Analyses

The purpose of the statistical analysis was to compare the hazard of HIV acquisition among those exposed to HCT to those unexposed to HCT. I first explored descriptive relationships in the data. I calculated the crude incidence rates within each exposure group and calculated a crude hazard ratio. I informally examined plots of the hazards as a function of each time scale to assess whether hazards were constant over time and hazard ratios were proportional over time. An interaction term with time was assessed formally using a likelihood ratio test. The model with the interaction term can be expressed as follows.

[3] $h_i(t) = h_o(t) * e^{\beta 1 HCT + \beta 2 HCT * time}$

Addressing Confounding

There were several socio-economic and behavioral covariates that were explored as potential modifiers and confounders of the relationship between HCT and HIV acquisition. Age and gender were explored as potential modifiers and included if they differed at an alpha level of 0.1. Otherwise they were to be included as confounders. Other confounders included in the analysis were education, whether sexual debut had occurred, whether a woman had ever been pregnant or a man had ever fathered a child, number of sexual partners in the last year, and condoms us at last sex.

Because this analysis included time-varying covariates whose values in one time period were potentially affected by their exposures in a previous time period, traditional statistical methods could not be used. Marginal structural models were needed [41]. In marginal structural models, a pseudo-population was created by using inverse probability of exposure weights, which are estimated similarly to propensity scores and amount to a parametric standardization of the data [42].

Accounting for Selection Bias

As described earlier, I explored the data for informative selection and attempted to correct for it. The directed acyclic graph in Figure 3.3 shows the structure of selection bias in this analysis. One frequently used set of criteria for informative selection are 1) that the proportion not participating is substantial, 2) that participation is associated with the exposure (HCT) and 3) that participation is associated with the outcome (HIV status) [37].

The first criterion is met. Approximately half of those eligible for the sero-survey refused to provide a sample in the ACDIS sero-survey at each time point [25]. For the second criterion, it was possible to assess the relationship between participation and prior HCT. This is because there were many persons who participated in ACDIS and answer questions about previous HIV testing, but refused to provide blood for HIV testing. For the third criterion, it was possible to assess

whether participation was associated with HIV status. This can be done by exploring whether those found to be HIV positive in one time period were more or less likely to participate in a subsequent time period, especially those who knew their HIV status. This type of assessment was conducted in a Malawian longitudinal cohort. It was found that persons who were HIV-positive and knew their HIV status were four times less likely to participate than those who were HIV-negative and knew their status [43-44]. Research in this cohort suggests that those who refused to be tested for HIV were more likely to die than those who did not [29]. This suggests that participation is associated with the outcome. However, given that all persons in this analysis started out HIV-uninfected, death from AIDS was unlikely during the follow-up period, even among persons who may have acquired HIV.

Refusal was a more plausible source of selection bias. We addressed loss to follow-up by refusal through inverse probability weighting (IPW). This method assigns a weight to each selected subject so they account not only for themselves but also for missing participants with similar covariate patterns. For example, if five members of the study with a common covariate pattern are eligible for the sero-survey and two of these five (2/5) refused, the remaining three (3/5) would have an inverse probability weight of 5/3. This process results in a pseudo-population in which the two subjects who were lost are replaced by the three who remain [37]. This method was feasible in this cohort, as there was rich covariate information for many who did participate in ACDIS, but did not participate in the sero-survey. It is worth pointing out that IPW requires a missing at random (MAR) assumption. Thus, if true HIV-status influences participation or is associated with unknown or unmeasured covariates, this would result in biased estimation of the outcome. If those who participate are sound representations of those who are lost, then this method is sufficient.

Power and Sample Size

In this analysis, the working hypothesis was that HCT leads to a modest increase in HIV acquisition. A priori I assessed how much power there would be to detect hazard ratios ranging from 1 to 1.5 within each strata of gender. The cohort is dynamic with participants constantly entering and leaving. However, it was reasonable to assume that 8000 participants would meet eligibility criteria and contribute four years of person time from 2006-2010 [25]. Next, I assumed that the hazard of HIV acquisition was constant across time. Finally, I assumed that the number exposed and unexposed to HCT were equal. This was realistic because in the earlier years, most participants were "unexposed" to HCT and in later years, most participants were "exposed" to HCT. This assumption was varied to assess whether power calculations were sensitive to group sizes.

Looking at the entire population of men and women together, when the reference hazard was 2.5 per 100 person-years, there was 91% power to detect a hazard ratio of 1.25. If there were twice as many participants in the exposed or unexposed groups, there was still at least 85% power to detect a hazard ratio of 1.25.

Figure 3.5 displays the amount of power available to detect hazard ratios form 1 to 1.5 assuming reference hazard levels of 2, 2.5, and 3 sero-conversions per 100 person years. If the reference hazard were 3 per 100 person years (which is likely for women) there would be 83% power to detect a hazard ratio of 1.30 and 92% power to detect a hazard ratio of 1.35. Holding the number of people constant, but varying the amount of person-time in each exposure group to 2:1 or 1:2, there was still at least 80% power to detect a hazard ratio of 1.35. If the reference hazard were 2 per 100 person years, which is likely for men, there would 70% power to detect a hazard ratio 1.30 and 82% power to detect a hazard ratio of 1.35. This suggests that if findings in this study had been comparable to what has been seen in other studies, small significant differences would be detectable.

Aim 3

In Aim 3 I developed a framework to formally characterize nine possible combinations of awareness of HIV status within HIV-discordant couples. This aim was designed to be descriptive, rather than analytic. I describe the framework, as well as its implications for interpreting existing research and potential uses for framing future research. A brief synopsis of the framework is described below

In HIV-discordant dyads, when both the HIV-infected member and the HIV-uninfected members have tested and disclosed, mutual awareness of HIV status ensues. Mutual awareness within HIV-discordant couples is one possible "awareness pattern." In addition, based on various combinations of awareness of one's own HIV status and one's partner's HIV status, there are eight other patterns. These nine patterns are outlined in Table 3.2. In one extreme scenario (1), neither the HIV-positive nor the HIV-negative partners know even their own HIV status. In the other extreme scenario (9) both partners know their own HIV status and both have disclosed it to the other. The other scenarios (2 through 8) reflect intermediate combinations of awareness. This same table can be replicated for couples with concordant HIV-positive or concordant HIV-negative statuses. However, I focus on HIV-discordant dyads (however stable) because this is the setting where there is risk for HIV transmission.

This framework has implications for understanding prevention research, including the results of HIV-discordant couple trials. Most trials, for ethical reasons, recruit couples who are all in pattern 9 [2, 45]. If this is the most protective scenario, results may be less generalizable to couples in the other eight scenarios. When results are implemented in couples in the other scenarios, results may change.

Understanding risk within each awareness pattern is important, and there are ways to study this. There are several large, longitudinal household-based surveys throughout sub-Saharan Africa that may be able to address this question observationally [46]. To do this would require first determining which couples were HIV-discordant. This could be done through ascertainment

of individual HIV status combined with household based information linking couples together [47]. Next, it would be necessary to determine what proportion of these couples were in each awareness pattern 1through 9. This would require capturing information on each individual's HCT care-seeking and partner-disclosure behaviors. Finally, these couples would need to be followed over time for intermediate outcomes (sexual behavior) and ultimate outcomes (HIV sero-conversion).

Understanding how the prevalence and protectiveness each of these patterns is is important for a more nuanced understanding of HIV prevention. It is also important for determining how best to optimize HIV prevention. The framework , along with its implications, are described in more detail in Chapter 6.

Tables and Figures

Table 3.1: Timing of Exposure and Outcome Assessment in ACDIS

Question	Response Options	2004	2005	2006	2007	2008	2009	2010	2011
a) Do you know your status from previous HIV testing?	Yes/No/ Refused			X	X	X	X	X	X
b) Provided blood for sero-testing		X	X	X	X	X	X	X	X

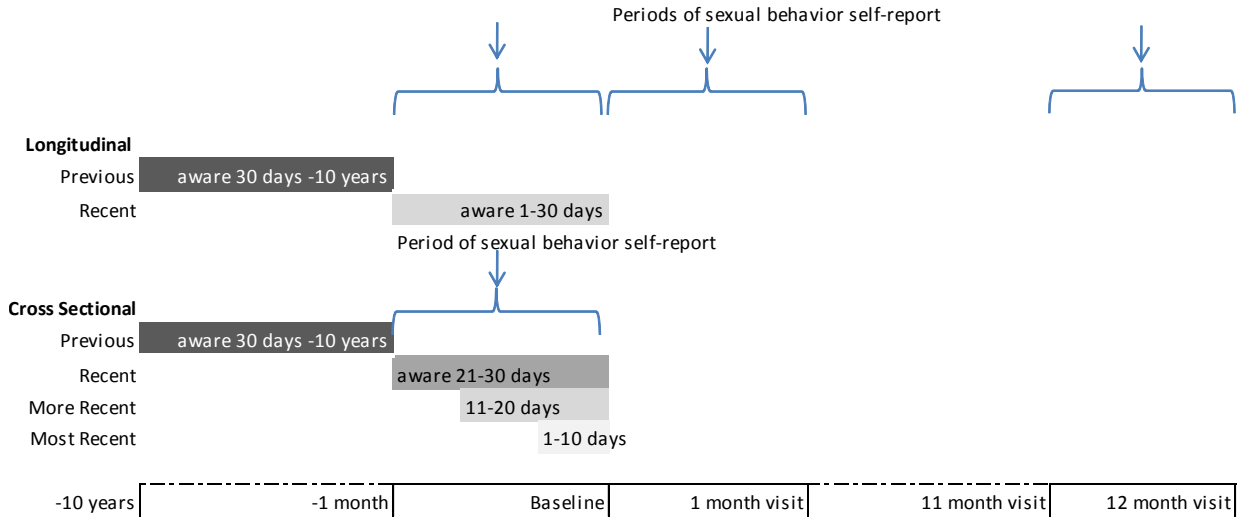
Caption: Figure 3.1 shows the time points when the exposure and outcome were ascertained.

Table 3.2: The Awareness Framework

HIV- partner	**HIV+ partner**		
	Not aware of own status	Aware of own status, has not disclosed	Aware of own status, has disclosed
Not aware of own status	1	2	3
Aware of own status, has not disclosed	4	5	6
Aware of own status, has disclosed	7	8	9

Caption: Table 3.2 is a preliminary depiction of the Awareness Framework. The Awareness Framework is described in more detail in Chapter 6.

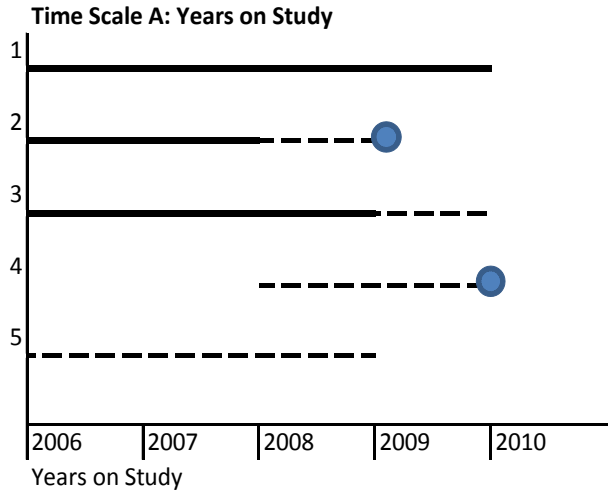
Figure 3.1 Schematic for Aim 1

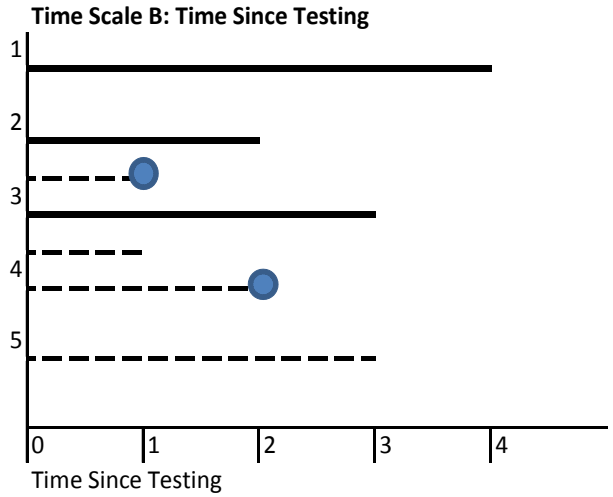


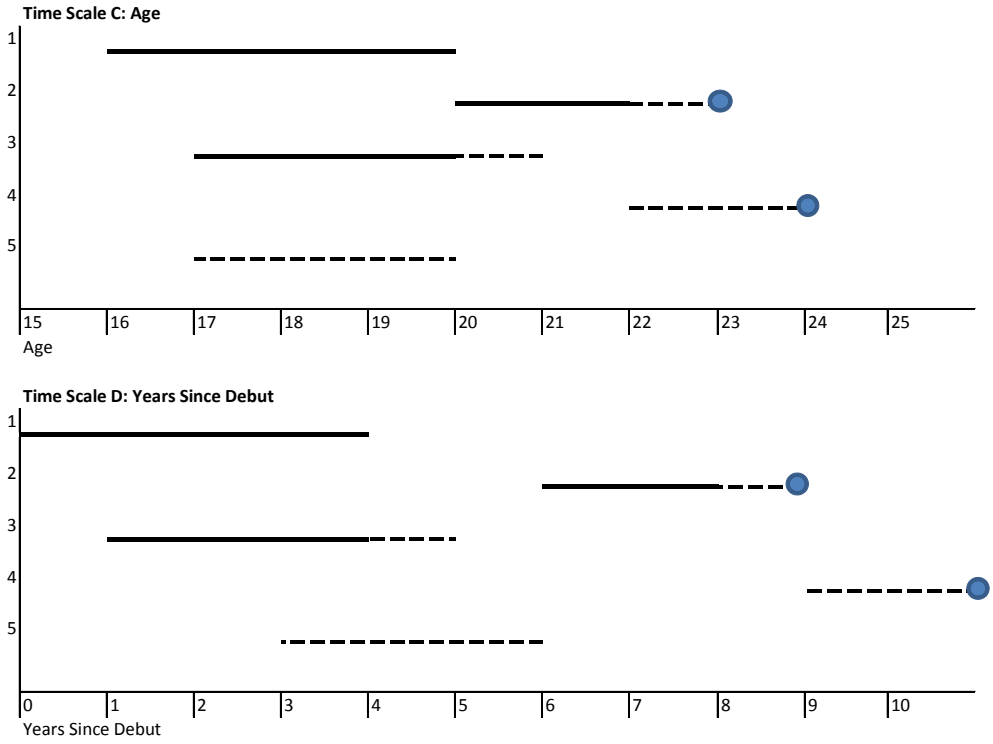
Caption: Figure 3.1 shows the exposure groups and one-month periods when outcomes were assessed in the longitudinal and cross-sectional analyses. In the cross sectional analysis, the recently aware period is divided into three exposure categories to see if there is a monotonic relationship between how long someone was aware of their HIV status and unprotected sex. In the longitudinal analysis, the cut-point for recent diagnosis is <30 days. At basleine those in the "recent" group were aaware of their HIVstatus for only part of the month before baseline, whereas those in the "previous" group were aware of their HIV status for the entire month preceding baseline. By the one and twelve month visits, persons in all groups were aware of their HIV status for the entire month preceding the visit.

Figure 3.2 Examination of a Small Risk Set Using Four Different Time Scales

Participant	1st visit	Censored	Tested	Birth Year	Debut	PYs. ~exp.	PYs exp.	Event
1	2006	2010	-	1990	2006	0	4	-
2	2006	-	2008	1986	2000	2	1	2009
3	2006	2010	2009	1989	2007	0	2	-
4	2008	-	2008	1986	1999	0	2	2010
5	2006	2009	2006	1989	2003	0	3	-

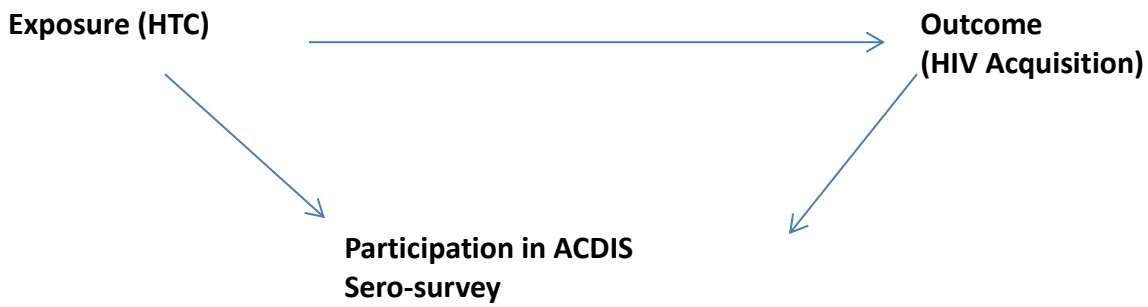






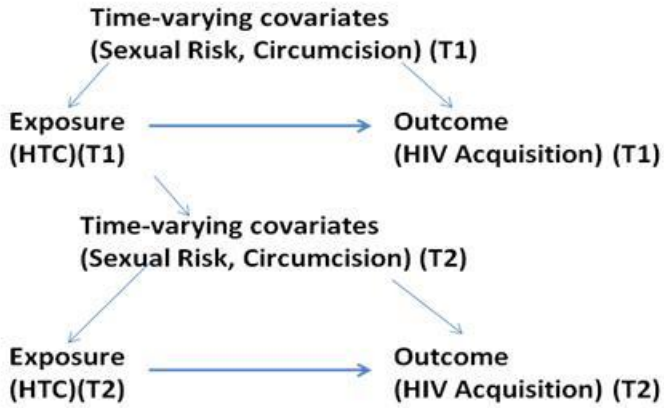
Caption: Figure 3.2 displays the same five hypothetical participants on four different time scales. Solid lines represent person-time when participants had not received HCT. Dotted lines represent person-time after participants had received HCT. Circles represent HIV sero-conversion events. On all four time scales, the participants contribute the same number of person-years to each exposure category. They also experience the same number of events. However, when an event occurs the participants in the risk set differ. In Aim 2, the primary time scale is time since HIV testing. Of note, persons who experience an event after testing for HIV are compared to themselves before receiving HCT. They are in their own risk set. Other relevant time scales were adjusted for through the use of covariates. In addition, the hazard of HIV acquisition was explored on the timeon study time scale.

Figure 3.3: Causal Structure of Selection Bias



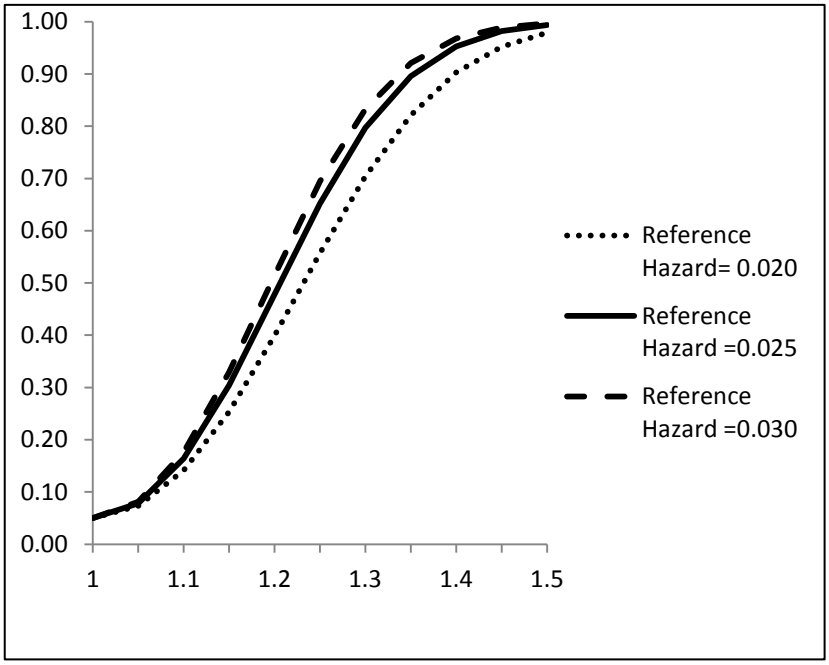
Caption: Figure 3.3 is a causal diagram depicting the simple structure for selection bias as it relates to the Aim 2 analysis.

Figure 3.4: A Causal Structure of Time Varying Confounding



Caption: Figure 3.4 is a causal diagram simplifying the causal structure of time-varying confounding affected by a prior exposure. This is one structure that lends itself to the use of marginal structural models for valid estimation of the total effect.

Figure 3.5: Power Calculation for Aim 2



Caption: Figure 3.5 depicted the expected amount of power available to detect hazard ratios form 1 to 1.5 assuming reference hazard levels of 2, 2.5, and 3 sero-conversions per 100 person years. If the reference hazard were 3 per 100 person years (which was expected for women) there was 83% power to detect a hazard ratio of 1.30 and 92% power to detect a hazard ratio of 1.35. Holding the number of people constant, but varying the amount of person-time in each exposure group to 2:1 or 1:2, there was still at least 80% power to detect a hazard ratio of 1.35. If the reference hazard were 2 per 100 person years (which was expetected for men) there was 70% power to detect a hazard ratio 1.30 and 82% power to detect a hazard ratio of 1.35. This suggests that if findings in this study were comparable to what was seen in other studies, small significant differences would be detectable.

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CHAPTER IV: Aim 1

Couples HIV Testing and Counseling Leads to Immediate, Sustained Consistent Condom Use among South African Stable HIV-discordant Couples

Introduction

Within stable HIV-discordant couples, HIV-uninfected partners are at ongoing risk for HIV acquisition [1]. In sub-Saharan Africa, stable HIV-discordant couples account for as few as 14% to as many as 94% of new infections [2-3]. Even if the fraction of new infections is on the lower end of the range, the absolute number of persons acquiring HIV from stable HIV-infected partners is substantial, given 1.8 million annual infections in sub-Saharan Africa [4].

Within HIV-discordant couples, antiretroviral therapy taken by HIV-infected partners or pre-exposure prophylaxis taken by HIV-uninfected partners can reduce HIV incidence [5-7]. However, not all HIV-discordant couples are eligible for biomedical interventions, and in the short-term, these interventions may not reach most HIV-discordant couples.

Behavioral interventions, such as couples HIV counseling and testing (HCT) remain viable approaches to prevention within HIV-discordant couples. Couples HCT is associated with increased condom uptake [8-12]. However, timing of condom uptake is not well understood, nor is whether those who continue engaging in unprotected sex engage in fewer unprotected acts.

The objective of this analysis is to assess whether HIV-infected persons in stable HIV-discordant couples increase condom uptake immediately after HCT and whether this behavior is maintained. We also assess the impact of HCT on number of sexual acts among persons who continue to engage in unprotected sex. To assess these questions we use behavioral data from South African HIV-discordant couples enrolled in Partners in Prevention HSV/HIV Transmission Study [13].

Methods

Participants

Partners in Prevention HSV/HIV Transmission Study was a randomized placebo controlled trial to assess the impact of acyclovir taken twice daily by HIV-1/HSV-2 coinfected persons on HIV-1 disease progression [14] and HIV-1 transmission to HIV-1 uninfected sex partners [13]. Couples were followed for up to 24 months from 2004-2008 or until death, drop-out, or site closure. This analysis uses data from 508 HIV-infected participants enrolled in the South African sites: Gugulethu, Orange Farm, and Soweto. Three HIV-infected participants enrolled with two HIV-uninfected partners, but for this analysis, only sexual behavior of the first HIV-uninfected partner is analyzed.

Couples were screened to identify which were HIV-discordant and eligible. HIV-infected participants were eligible if they had CD4 $\geq$250, no AIDS-defining illness, and were HSV-2 seropositive. Couples who did not expect to remain together $\geq$24 months and those with no sexual activity for $\geq$3 months were excluded. Detailed descriptions of recruitment, eligibility, and baseline characteristics are available [13-16].

Ethical Approval

The trial was approved by the Human Subjects Review Committee at University of Washington, and ethical review committees at participating sites. This analysis was approved by the Public Health-Nursing Institutional Review Board at University of North Carolina, Chapel Hill.

Behavioral Interventions

Many HIV-infected participants, especially in Soweto, had initially been tested through individual provider- or client-initiated HCT months or years before study entry. These participants also received couples HCT shortly before study entry. Other HIV-infected participants had learned their HIV status for the first time within days or weeks of study entry through couples HCT. Thus, by baseline all HIV-discordant couples had participated in couples HCT. Couples HCT emphasized the risk for HIV transmission within the couple, as well as risk reduction through abstinence or consistent condom use. Additionally, HIV-infected participants were counseled and provided with free condoms monthly. HIV-uninfected participants also received HCT quarterly, typically with the HIV-infected partner.

Data collection

Trained research staff collected demographic and sexual behavior information at baseline and monthly follow-up using interviewer-administered questionnaires.

Factor of Interest and Outcome Assessment

The primary factor of interest was timing of HCT for the HIV-infected participant. At baseline, HIV-infected participants reported the date of their first HIV-positive test. This date was subtracted from the baseline date to determine the number of days since HCT. Some HIV-infected participants had been tested >30 days before baseline (previously tested). Others learned their HIV status <30 days before baseline (newly tested). For some analyses, the newly tested group was further divided into three categories: HCT $\leq$7, 8-14, and 15-30 days before baseline. We explored cut-points within the previously tested category, but did not observe improved model fit when a term for HCT >60 days (p=0.7) or >365 days (p=0.3) was included.

The primary outcome was unprotected sex self-reported by the HIV-infected participant. At baseline and each month thereafter, HIV-infected participants were first asked the total

number of vaginal and anal sex acts they had with their study partner in the last month and, of those acts, the number of times a condom was used. From these responses, numbers of sex acts and unprotected sex acts in the last month were calculated.

At baseline, for previously tested persons (>30 days), all unprotected sex acts must have occurred *after* HCT. For newly tested participants, unprotected sex could have occurred *before* becoming aware of their HIV status or soon after. Persons tested ≤7 days before baseline were unaware of their HIV status for most of the month preceding baseline and serve as a proxy for persons unaware of their HIV-positive status. At all subsequent visits, all HIV-infected participants were aware of being HIV-infected and in HIV-discordant relationships for the full month preceding the visit (Figure 4.1).

We compared the sexual behavior of newly and previously tested persons at baseline and months one, six and twelve. We hypothesized that at baseline the persons tested ≤7 days before baseline would have the highest prevalence of unprotected sex and the highest number of unprotected acts, but by month one all groups would be comparable.

Covariates

A directed acyclic graph was used to identify possible confounders of the association between time since HCT and unprotected sex [17]. Individual-level variables were gender, age, education, having a living child, having ≥1 sex partner in the previous month (including ≥1 study partner), and study site. Couple-level variables were marital and cohabitation status, relationship length, relationship violence in the past 3 months, and male-female age difference.

Baseline Analyses

Number of unprotected sex acts is a count. At baseline, we explored the relationship between time since HCT and number of unprotected sex acts in several types of models for count data: Poisson, negative binomial (NB), zero-inflated Poisson (ZIP), and zero-inflated negative

binomial (ZINB). A likelihood ratio test was conducted to determine whether data were over-dispersed, a scenario when an NB model is a better fit for the data than a Poisson model. A Vuong test was conducted to determine whether a zero-inflated model was a better fit for the data than a simple NB or Poisson model [18]. These tests indicated that the ZINB model provided the best model fit.

By solving two simultaneous equations, ZINB models generate two sets of parameters [18]. The first set, generated using a logistic regression procedure, estimates the odds of being in a group that can only get a zero count (i.e. zero unprotected sex acts in the last month) between two exposure groups. The second set, generated using a Poisson or NB procedure, models the relative number of unprotected sex acts between the exposed and unexposed, conditional on not being in the first group. To mitigate influence of extreme observations and facilitate model convergence, participants with >15 sex acts (N=20, median number of acts=25) were re-coded with 16 sex acts.

We implemented unadjusted and adjusted analyses. To determine which variables to include in the final adjusted analysis, we first ran a model with all covariates presented in Table 4.1 and interaction terms for age, gender, and site. Interaction terms were retained if they reached statistical significance at alpha=0.1. Covariates were removed one-by-one, and retained if removal resulted in >10% change in either of the parameter estimates comparing $\leq$7 days to >30 days [19-20]. A fully adjusted model was implemented as a sensitivity analysis.

Longitudinal Analyses

We used generalized estimating equations (GEE) to assess the effect of HCT timing on sexual behavior at baseline, and months one, six, and twelve among couples that remained HIV-discordant. The month one time point was selected to assess whether behavior change occurred soon after HCT and the month six and twelve time points were selected to assess whether behavior change was maintained. Logistic models were used in the entire population and NB

models were restricted to persons reporting ≥1 unprotected sex act in a given period. In both logistic and NB models, to account for within subject correlation, robust variance estimators with exchangeable correlation matrices were used [21]. We calculated odds ratios (OR), relative numbers of unprotected acts, predicted probabilities of unprotected sex, predicted numbers of unprotected sex acts, and 95% confidence intervals (CI) in each group. We used the same model-building strategy described above to arrive at final models. Fully adjusted models were implemented as sensitivity analyses.

Both baseline and longitudinal models were restricted to persons sexually active with their study partners. Analyses were conducted in SAS v.9.2. (SAS Institute, Cary, North Carolina).

Results

Descriptive Statistics

Soweto was the most common enrollment site (47%), followed by Gugulethu (39%), and Orange Farm (14%) (Table 4.1a). Most HIV-infected participants (77%) were female. The mean age of HIV-infected participants was 33 years and 29% had completed secondary school. Most HIV-infected participants (82%) had at least one child and few (4%) reported >1 sex partner in the last month. Two thirds of couples were married or cohabitating; 79% had been together for >1 year (Table 4.1b). On average, males were 4.1 years older than females, regardless of which partner was HIV-infected. Few HIV-infected participants (4%) reported recent relationship violence.

At baseline, 13% of HIV-infected participants were tested ≤7 days before baseline, 26% 8-14 days before baseline, 11% 15-30 days before baseline, and 50% >30 days before baseline (Table 4.1a). The median time since HCT was 29 days (IQR: 11 days, 9.2 months) overall and 9.2 months (IQR: 3.8 months, 25.6 months) among the previously tested.

At baseline, almost all HIV-infected participants reported ≥1 sex act in the last month with their study partner (new: 94.1%, previous: 96.1%, p=0.3) (Table 4.2). Among those newly tested, 53% reported ≥1 unprotected sex act in the last month compared to 25% of those previously tested (OR: 3.3, CI: 2.3, 4.8). Of those reporting any unprotected sex, the mean numbers of unprotected acts were eight (newly tested) and six (previously tested).

One month after baseline, most HIV-infected participants reported sexual activity with study partners (new: 87.9%, previous: 89.1%, p=0.7). Nine percent of those newly tested and 13% of those previously tested reported any unprotected sex in the last month (OR: 0.7, CI: 0.4, 1.3). Of those reporting any unprotected sex, the mean numbers of unprotected acts were eight (newly tested) and seven (previously tested).

Twelve months after baseline, most HIV-infected participants continued to report sexual activity with study partners (new: 73.6%, previous: 78.2%, p=0.3). Six percent of those newly tested and 14% of those previously tested reported any unprotected sex in the last month (OR: 0.4, 95% CI: 0.2, 0.8). The mean number of unprotected sex acts was 6 in both groups.

Baseline Analyses

In bivariable analysis, both the zero-inflated odds of unprotected sex and the number of unprotected sex acts in the month prior to enrolment were higher among those tested ≤7 days before baseline compared to those tested >30 days before baseline [OR: 9.0 (CI: 3.4, 23.9); relative number: 1.7 (CI: 1.2, 2.6)]. The odds of engaging in unprotected sex were also higher among those tested 8-14 days and 15-30 days before baseline compared to those tested >30 days before baseline, with an apparent dose-response relationship. Number of unprotected acts was also higher in these groups, compared to those tested >30 days before baseline (Table 4.3).

In multivariable analysis with adjustment for study site and marital status, the odds of being in a group engaging in unprotected sex were higher among those tested ≤7 days before baseline compared to those tested >30 days before baseline [aOR: 9.3 (CI: 3.6, 24.2)]. Similarly,

the number of unprotected sex acts was higher among those tested ≤7 days before baseline compared to those tested >30 days before baseline [adjusted relative number: 1.7 (CI: 1.2, 2.6)]. The final adjusted model differed minimally from the fully adjusted model (2%, logistic parameter estimate; 8%, NB parameter estimate). Both the adjusted odds and adjusted relative number of unprotected sex acts were higher among those tested 7-14 and 15-30 days before baseline compared to those tested >30 days before baseline (Table 4.3).

Longitudinal Analyses

At baseline, the odds of unprotected sex in the last month were substantially higher among those tested ≤7 days before baseline than those tested >30 days before baseline [OR 7.01 (CI: 3.80, 12.94)], but these groups were similar by month one [OR 0.53 (CI: 0.18, 1.58)] and remained similar at months six [OR 0.45 (CI: 0.15, 1.34)] and twelve [OR 0.40, CI (0.10, 1.53)].

Within each group, the odds of any unprotected sex were lower at month one than at baseline [≤7 days OR 0.03 (CI: 0.01, 0.11), 8-14 days OR 0.09 (CI: 0.04, 0.19), 15-30 days OR 0.19 (CI: 0.08, 0.46), >30 days OR 0.45 (CI: 0.32, 0.64)]. Similarly, in all groups, the odds of unprotected sex were lower at months six and twelve compared to baseline [Month 6 versus baseline: ≤7 days OR 0.04 (CI: 0.02, 0.13), 8-14 days OR 0.17 (CI: 0.10, 0.31), 15-30 days OR 0.16 (CI: 0.06, 0.46), >30 days OR 0.69 (CI: 0.48, 1.00)] [Month 12 versus baseline: ≤7 days OR 0.04 (CI: 0.01, 0.14), 8-14 days OR 0.10 (CI: 0.05, 0.23), 15-30 days OR 0.06 (CI: 0.01, 0.32), >30 days OR 0.66 (CI: 0.47, 0.94)] (Figure 4.2a.) Model-building resulted in no adjustment variables, so results are not presented. Full adjustment resulted in a 7% change on the primary comparison of interest (month one versus baseline among persons tested ≤7 days before baseline).

In longitudinal NB analysis, all newly tested participants (<30 days) were analyzed together due to sparse data. The number of unprotected sex acts was higher among the newly tested than the previously tested at baseline [1.4 (CI: 1.1, 1.8)] but the groups were the same by

month one [relative number: 1.0 (CI: 0.6, 1.8)] and remained so at month six [relative number 0.9 (CI: 05, 1.7)] and twelve [relative number: 1.1 (CI: 0.6, 2.0)].

Among the newly tested, the number of unprotected sex acts in the last month was similar at months one [0.8 (CI: 0.5, 1.3)], six [0.7 (CI: 0.4, 1.1)], and twelve [1.0 (CI: 0.6, 1.7)] compared to baseline, but results were imprecise. Among previously tested persons, the number of unprotected sex acts was the same at months one [1.1 (CI: 0.9, 1.5)], six [1.0 (CI: 0.7, 1.5)], and twelve [1.2 (CI: 0.9, 1.7)] compared to baseline, though also imprecise (Figure 4.2b). Model-building resulted in no adjustment variables, so results are not presented. Full adjustment resulted in a 6% change in the primary comparison of interest (month one versus baseline among newly tested persons).

Discussion

The proportion of HIV-infected persons engaging in unprotected sex with their study partners declined considerably in the month after couples HCT, suggesting couples HCT facilitated rapid behavior change in these stable HIV-discordant couples. At baseline HIV-infected persons with previous HCT reported much lower levels of unprotected sex in the last month (26%) than persons who had received HCT $\leq$7 days before (71%). By month one, once both groups had received couples HCT, both reported lower, comparable levels of unprotected sex (>30 days: 14%, $\leq$7 days: 8%). These levels persisted for one year (>30 days: 19%, $\leq$ 7 days: 8%).

The protective nature of couples HCT for HIV-discordant couples is consistent with findings from earlier work in Africa. Couples HCT is associated with high condom uptake among HIV-infected persons [22-23], particularly persons in HIV-discordant relationships [8-12]. Our analysis is one of the first to show that condom uptake occurs within the first week after couples HCT [9].

A modest decline in unprotected sex was observed from baseline to month one among HIV-infected persons who had received HCT previously. Although these persons had sought HCT before, some may not have disclosed their HIV status to sex partners, learned their partner's HIV status, or received counseling with their partners until just before baseline. This decline suggests mutual awareness of HIV-discordance is more protective than individual awareness of HIV infection. This interpretation is complemented by findings from the full trial: HIV-uninfected participants reported less frequent unprotected sex with study partners, whose HIV status was known, than with outside partners, whose HIV status was often unknown [24].

After baseline, all HIV-infected persons received individual counseling monthly, partners received individual or couples HCT quarterly, and condoms were provided. These factors may have contributed to ongoing consistent condom use, though it cannot be determined how influential these factors were compared to the initial impact of couples HCT.

In spite of couples HCT and ongoing counseling, some HIV-infected persons continued engaging in unprotected sex with study partners, without reducing the number of unprotected acts. Understanding the reasons for ongoing risk and the acceptability of other prevention strategies, including biomedical strategies, is essential.

Understanding the impact of HCT on HIV prevention is critical given its rapid scale-up. However, HCT is difficult to assess in randomized settings because withholding HCT is unethical and observational studies are typically subject to confounding. This trial provided an opportunity to address the impact of couples HCT on HIV prevention in an ethical, rigorous way. We believe our results are unlikely to be heavily biased by unmeasured confounding because the main difference between exposure groups was the timing of HCT and study enrollment. This typically differed by only a few months and *a priori* seems unlikely to be strongly influenced by social or biomedical factors. The similar distribution of observed covariates between exposure groups (Table 4.1) and the need for minimal adjustment support this contention.

In our study, we knew the precise timing of when someone learned their HIV status, but could only determine when sexual behavior occurred within a one-month interval. The discrepancy in the timing of these measures leaves ambiguity regarding the temporal order of HCT and sexual behavior for newly tested persons. For example, someone tested 10 days prior to baseline spent the first 20 days of the month unaware of their HIV status and the final 10 days aware. If they reported unprotected sex during this thirty-day period, it could have occurred before, after, or both before and after HCT. Two features of our study design lend evidence to the strong possibility that unprotected sex occurred predominantly *before* HCT. First, once newly tested persons had been aware of their HIV status for at least one month they reported lower levels of unprotected sex. Second, at baseline, the relationship between the amount of time someone was unaware of their HIV status and the odds of unprotected sex was monotonic. The more time someone spent unaware of their HIV status, the more likely they were to report any unprotected sex at baseline. If this trend were to continue, a group unaware for the entire month would be expected to experience an even higher probability of unprotected sex relative to the previously tested.

This analysis relied on self-report which is subject to social desirability. If persons were more likely to over-report condom use after HCT than before, effect measures would be exaggerated. Biomarkers suggest that these differences are unlikely to be explained entirely by this concern. In the full trial population, although consistent condom use was likely over-reported, it was strongly associated with reductions in HIV acquisition [25].

Caution is needed when generalizing results beyond these stable HIV-discordant couples. Persons enrolling in HIV prevention trials may be more motivated to adopt HIV prevention behaviors than the general population. Additionally, persons who are willing to enroll with partners may differ from persons who are unwilling. Most couples were in long-term marital or cohabiting relationships and levels of intimate partner violence were low [26]. Couples HCT may not be as protective in segments of the population in less stable, more violent partnerships[27].

Understanding effectiveness of couples HCT in these less stable partnerships is an area for future investigation.

Our findings raise questions about the current HCT paradigm, which is not typically couple-oriented. When stable couples learn that they are in HIV-discordant relationships they adopt consistent condom use quickly, but such marked behavior change is not typically reported after individual HCT [28-29]. Couples HCT assures simultaneous disclosure, has a substantial impact on sexual behavior [29] and may have an impact on adherence to biomedical prevention [7, 30]. However, most current HCT efforts are aimed at individuals, not couples, leading to missed HIV prevention opportunities. Strategies, such as home-based testing [31], supportive HIV-disclosure counseling [32], voucher-based recruitment [33], and provider-assisted partner notification [34] can be used to inform persons of HIV-discordance. These strategies, which are feasible, acceptable, effective, and cost-effective, must be public health priorities.

In summary, our results add to a growing body of evidence demonstrating that couples HCT is effective at rapidly increasing condom-uptake, facilitating ongoing condom use, and possibly lowering rates of HIV acquisition [10-11, 25]. Although initial findings were published nearly two decades ago, most countries have been slow to implement couple-based strategies. With expanding HCT capacity in Africa [35], decision-makers now need to consider how to reach couples. Such expansion will help a high risk group make informed sexual health decisions and likely prevent a substantial number of infections.

Tables and Figures

Table 4.1a Baseline characteristics of HIV-infected participants by time since HCT

Characteristics	Newly Tested						Previously Tested	
	≤7 days		8-14 days		15-30 days		>30 days	
	N=65	(13%)	N=134	(26%)	N=55	(11%)	N=254	(50%)
Gender								
Male	14	(21.5)	31	(23.1)	16	(29.1)	58	(22.8)
Female	51	(78.5)	103	(76.9)	39	(70.9)	196	(77.2)
Age*								
<25	12	(18.8)	18	(13.6)	4	(7.3)	48	(18.9)
25-34	27	(42.2)	53	(40.2)	35	(63.6)	132	(52.0)
≥35	25	(39.1)	61	(46.2)	16	(29.1)	74	(29.1)
Education								
<secondary	44	(67.7)	111	(82.8)	37	(67.3)	167	(67.7)
≥secondary	21	(32.3)	23	(17.2)	18	(32.7)	87	(32.3)
Has a living child								
Yes	55	(84.6)	100	(74.6)	44	(80.0)	218	(85.8)
No	10	(15.4)	34	(25.4)	11	(20.0)	36	(14.2)
>1 sex partner								
Yes	4	(6.2)	6	(4.5)	1	(1.8)	7	(2.8)
No	61	(93.8)	128	(95.5)	54	(98.2)	247	(97.2)
Study Site								
Gugu-lethu	24	(36.9)	79	(59.0)	30	(54.5)	62	(24.4)
Orange Farm	13	(20.0)	22	(16.4)	10	(18.2)	28	(11.0)
Soweto	28	(43.1)	33	(24.6)	15	(27.3)	164	(64.6)

*Results may not add to column totals due to missing data.

Table 4.1b Baseline characteristics of HIV-infected couples by time since HCT

Couple Characteristics	Newly Tested						Previously Tested	
	≤7 days		8-14 days		15-30 days		>30 days	
	N=65	(13%)	N=134	(26%)	N=55	(11%)	N=254	(50%)
Marital/Cohabitation Status								
Not married, not cohabitating	16	(24.6)	48	(35.8)	26	(47.3)	81	(31.9)
Not married, cohabitating	40	(61.5)	70	(52.2)	23	(41.8)	120	(47.2)
Married	9	(13.9)	16	(11.9)	6	(10.9)	53	(20.9)
Relationship length*								
<1 year	15	(23.1)	40	(30.1)	14	(25.5)	38	(15.1)
1-3 years	14	(21.5)	46	(34.6)	17	(30.9)	90	(35.9)
>3 years	36	(55.4)	47	(35.3)	24	(43.6)	123	(49.0)
Age Difference*†								
<5 years	35	(55.6)	70	(53.0)	32	(59.3)	134	(53.2)
≥5 years	28	(44.4)	62	(47.0)	22	(40.7)	118	(46.8)
Partner Violence ≤3 Months								
Yes	3	(4.6)	5	(3.7)	1	(1.8)	11	(4.3)
No	62	(95.4)	129	(96.3)	54	(98.2)	243	(95.7)

*Results may not add to column totals due to missing data.
†Male age minus female age regardless of which partner is HIV-infected

Table 4.2 Sexual Behavior in the Previous Month Comparing Newly to Previously Tested at Baseline, Month One, and Month Twelve

	Baseline (N=508)		1-Month (N=487)		12-Month (N=376)	
Timing of testing	New	Previous	New	Previous	New	Previous
Entire Population	n=254 (50%)	n=254 (50%)	n=239 (49%)	n=248 (51%)	n=174 (46%)	n=202 (54%)
Total sex acts						
N (%) ≥1	239 (94.1)	244 (96.1)	210 (87.9)	221 (89.1)	128 (73.6)	158 (78.2)
N (%) = 0	15 (5.9)	10 (3.9)	29 (12.1)	27 (10.9)	46 (26.4)	44 (21.8)
Mean (SD)	7.1 (10.3)	6.9 (9.3)	5.3 (6.5)	6.3 (7.7)	4.4 (5.3)	4.9 (5.6)
Median (IQR)	4 (3,8)	4 (3,8)	3 (2, 5)	4 (2, 8)	3 (0, 7)	3 (1, 7)
Unprotected sex acts						
N (%) ≥1	134 (52.8)	64 (25.2)	22 (9.2)	31 (12.5)	11 (6.3)	29 (14.4)
N (%) = 0	120 (47.2)	190 (74.8)	217 (90.8)	217 (87.5)	163 (93.7)	173 (85.6)
Mean (SD)	4.1 (9.7)	1.5 (5.1)	0.7 (3.8)	0.9 (4.4)	0.4 (2.1)	0.8 (3.2)
Median (IQR)	1 (0, 4)	0 (0, 1)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Persons with ≥1 unprotected act	n=134 (68%)	n=64 (32%)	n=22 (42%)	n=31 (58%)	n=11 (28%)	n=29 (73%)
Total sex acts						
Mean (SD)	9.4 (12.2)	7.5 (9.6)	13.1 (12.9)	10.2 (10.4)	9.0 (5.1)	7.8 (6.0)
Median (IQR)	5 (3, 12)	4.5 (2.5, 9)	7 (4, 20)	8 (4, 12)	8 4, 12)	7 (4, 9)
Unprotected sex acts						
Mean (SD)	7.8 (12.2)	5.8 (9.0)	7.7 (10.5)	7.1 (10.8)	6.1 (6.4)	5.7 (6.6)
Median (IQR)	4 (2, 9)	3 (1, 6)	2.5 (1, 8)	4 (2, 8)	2 (1, 12)	3 (2, 6)

SD=standard deviation, IQR=inter-quartile range

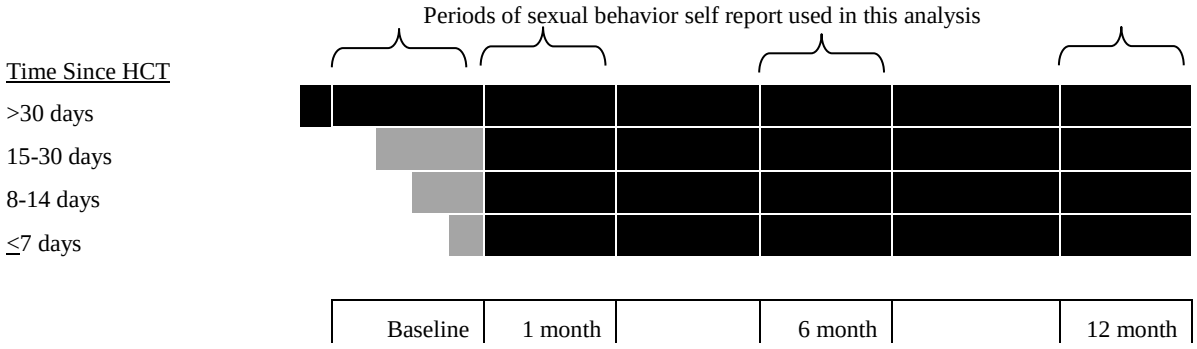
Table 4.3 Unadjusted and Adjusted Baseline ZINB Models by Time Since HCT

	Unadjusted Models		Adjusted Models*	
	OR of any unprotected sex (95% CI)	Relative # of unprotected acts (95% CI)	OR of any unprotected sex (95% CI)	Relative # of unprotected acts (95% CI)
Time Since HCT				
>30 days	1.	1.	1.	1.
15-30 days	2.6 (1.2, 5.4)	1.5 (0.9, 2.4)	3.2 (1.5, 7.0)	1.7 (1.1, 2.8)
8-14 days	3.5 (1.9, 6.4)	1.1 (0.8, 1.6)	4.2 (2.2, 8.0)	1.2 (0.8, 1.7)
≤7 days	9.0 (3.4, 23.9)	1.7 (1.2, 2.6)	9.3 (3.5, 24.2)	1.7 (1.2, 2.6)

ZINB=zero-inflated negative binomial; HCT=HIV counseling and testing; OR=odds ratio; CI=confidence interval

* Adjusted for marital status and study site

Figure 4.1 Schematic of Baseline and Longitudinal Analyses



Caption: Figure 4.1 displays the four exposure groups and time periods when outcomes are assessed. At baseline, those in the newly tested groups (≤7 days, 8-14 days, 15-30 days) are aware of their HIV status for only part of the month before baseline (indicated in gray), whereas those in the previously tested group (>30 days) are aware of their HIV status for the entire month before baseline (indicated in black). The more recently someone was tested, the longer they spent unaware of their HIV status. By months one, six and twelve, persons in all groups had known their HIV status for >30 days.

Figure 4.2a Unadjusted Predicted Probability of Any Unprotected Sex Acts (95% CIs) by Time since HCT

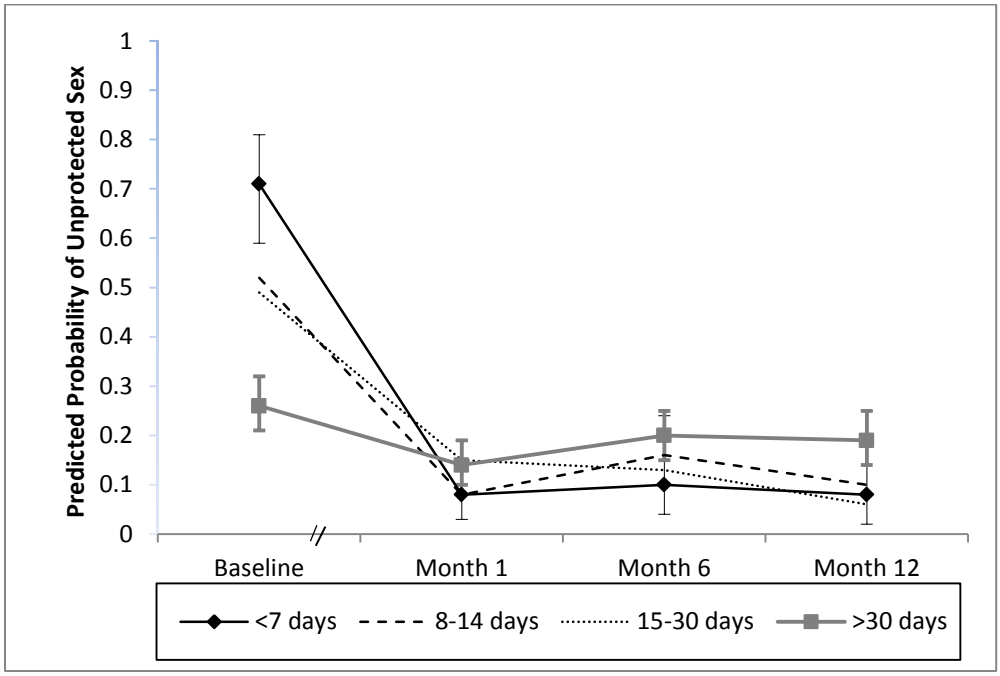
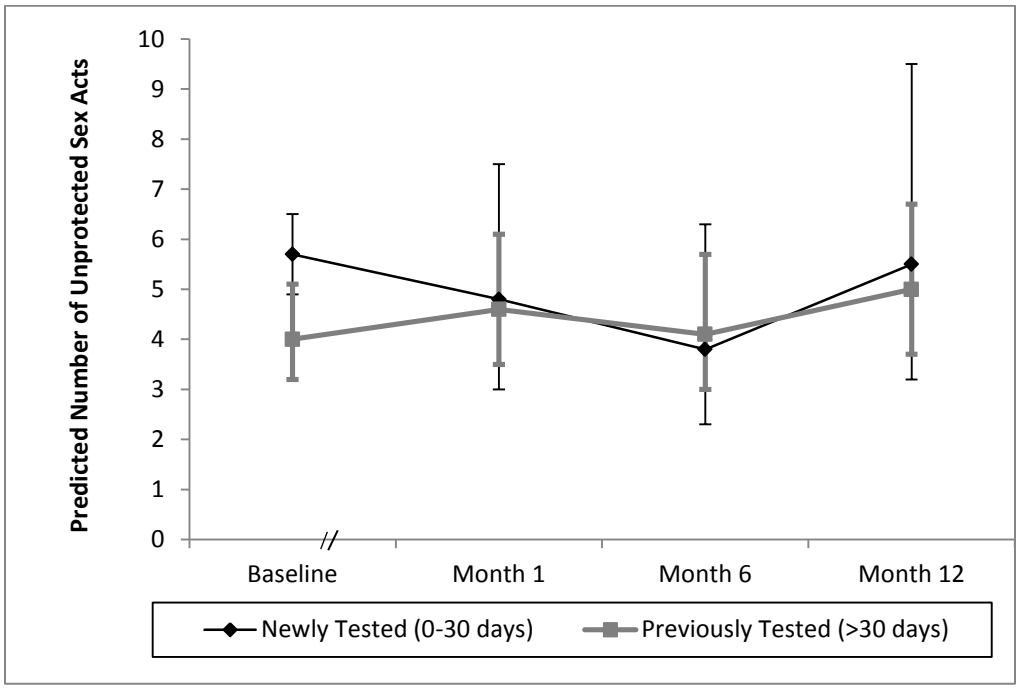


Figure 4.2b Unadjusted Predicted Number of Unprotected Sex Acts (95% CIs) by Time since HCT



Caption: Figure 4.2a depicts the predicted probability of unprotected sex among sexually active persons in all four groups over a one-year period. At baseline, the more recently someone had learned their HIV status, the higher the probability of unprotected sex. By month one, the predicted probability of unprotected sex declined in all four groups and remained lower over time. Figure 4.2b depicts the predicted number of unprotected sex acts among persons who engaged in unprotected sex at four time points over a one-year period. All newly aware groups are collapsed together due to sparse data. At baseline persons who were newly tested reported more unprotected sex acts in the last month than persons who were previously tested. One month after baseline, the predicted number of unprotected sex acts declined in the newly tested group and remained constant (though imprecise) in both groups over time.

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CHAPTER V: Aim 2

The Effect of HIV Counseling and Testing on HIV Acquisition among South African Youth: Evidence of Protectiveness

Introduction

Persons aged 15-24 years in sub-Saharan Africa are at high risk for HIV acquisition [1]. In South Africa, the country with the world's largest burden of HIV infection, an estimated 21% of girls and 5% of boys acquire HIV by the time they are 24 years old [2-4]. Youth in this age range urgently need effective HIV prevention interventions to slow HIV incidence.

In recent years, HIV counseling and testing (HCT) has been scaled up rapidly in sub-Saharan Africa [5-6]. From 2005 to 2008, the proportion of South Africans who ever sought HCT increased from 31% to 51% [2, 7]. HCT availability continued to increase in healthcare and other settings in South Africa in response to a national "know your status campaign," aimed at testing 15 million South Africans in a 14-month period from 2010-2011 [8]. South African youth tend to report lower uptake of HCT than adults [7, 9-10], in spite of the majority being sexually active by their mid-teens [4, 11]. To increase HCT uptake among this age group, introducing HCT into secondary schools has been debated [12-13], with the effect of HCT on incidence being one key component.

The impact of HCT on HIV acquisition has not been studied among youth. Studies in adults suggest HCT leads to no change or a modestly elevated HIV acquisition rate [14-15], a trend that may be due to behavioral disinhibition following testing [16], testers being higher risk people, or spurious statistical associations. The impact of HCT on HIV acquisition may be different among youth, as they are socially, and behaviorally different from adults [17]. Many

youth have not yet experienced sexual debut, and early behavioral interventions, including HCT, may be more effective during this formative period [18]. Sexually active youth are also not typically in marital or cohabitating unions [19], and condom initiation or continuation may be easier to negotiate in these types of relationships. An ongoing community-randomized study, Project Accept, is assessing the impact of intensive community-based HCT versus standard clinic-based HCT on community incidence among persons 16-32 in several developing countries, including South Africa [20]. However, understanding the impact of HCT on HIV acquisition at an individual level is necessary as well.

We conducted a retrospective cohort study using data from a large demographic surveillance area in rural South Africa [21] to compare HIV acquisition among youth who received HCT to those who did not from 2006-2011.

Methods

Setting

The study was conducted in Hlabisa, KwaZulu Natal, South Africa, a rural sub-district heavily affected by the HIV epidemic. HIV prevalence in this area is estimated at 23%, and HIV incidence rate is 3.4 per 100 person-years [22-24]. In 2005, HCT was introduced in all 17 primary health centers to support the scale-up of a decentralized HIV care and treatment program [25]. From 2005-2010, persons could voluntarily seek counselor-delivered HCT at these centers. Additionally, health providers in these centers referred all antenatal, sexually transmitted infection, and tuberculosis patients for the same counselor-delivered HCT with high uptake [26]. Home- and mobile- HCT were also available in some parts of the sub-district at a lower volume beginning in 2008, and these services were also counselor delivered [27]. All HCT services incorporate education about how HIV is transmitted, as well as counseling on sexual behavior risk reduction. All of these services use rapid tests so clients are provided with results on the day

they are tested. This analysis assesses the impact of all of these HCT services on HIV acquisition among youth using the data sources described below.

Study Design

Demographic characteristics were assessed semi-annually in an enumerated part of the Hlabisa sub-district through the Africa Centre Demographic Surveillance. Individual-level characteristics, such as age, gender, and education, were collected in this survey, as well as household-level characteristics, such as distance to the nearest primary health center.

In this same area, a separate annual population-based HIV Survey was conducted including a laboratory assessment of HIV status. For consenting participants, blood was obtained via finger prick and prepared into dried blood spots. A serial HIV testing algorithm with two ELISAs was used: Vironostika HIV-1/HIV-2 Microelisa System (Biomérieux, Durham, NC, USA), followed by Wellcozyme HIV 1+2 GACELISA (Murex Diagnostics, Benelux B.V., Breukelen, The Netherlands) [21]. The HIV Survey also assessed whether the participant had previously sought HCT [21] and self-reported sexual behavior.

For this analysis, in each year, one Demographic Surveillance record and one HIV survey record were merged together using a unique identifier. These annual records were combined so an individual's HCT history and HIV status could be assessed over time. We conducted a retrospective cohort study in this population.

Of note, the exposure (HCT self report) and the outcome (HIV status) were ascertained independently. On the day of the HIV survey participants reported whether or not they had sought HCT. They also provided blood for laboratory-based HIV testing. Participants did not learn their HIV status on the day of the HIV Survey. Therefore participants who reported being HCT-exposed learned their HIV status from one of the means described above prior to the survey.

Eligibility

To be eligible for this study, persons had to have at least two reports of HCT between 2006 and 2010 in the HIV survey, though they could have had up to five reports. At the first report, participants had to report *not* being aware of their HIV status from HCT and at the second report participants could either report being aware or not being aware of their HIV status from HCT. The midpoint between these two reports was considered.baseline. Those who reported HCT at the second report were considered HCT-exposed at baseline and those who reported no HCT at the second report were considered HCT-unexposed at baseline. Variables from first time point were used for adjustment with the baseline value. Some persons (20%) did not have HCT reports from two consecutive years (e.g. 2006 and 2007), so a report from a subsequent year is used as the second report (e.g. 2008). All persons in the study had to have at least two valid HIV test results from the HIV survey—one negative result before baseline and one negative, positive, or indeterminate result after baseline. The analysis was restricted to persons 15-24 years old at baseline.

Ethical Approval

Ethics permission for the demographic and HIV surveillance was obtained from the University of KwaZulu-Natal research ethics committee. The current analysis was approved by the Public Health-Nursing Institutional Review Board at the University of North Carolina, Chapel Hill.

Data Collection

All questions in the Demographic Surveillance were collected by a trained interviewer and reported by a household proxy. All questions on the HIV survey, including HCT-exposure and the sexual behavior assessment, were administered by a trained interviewer of the same sex

as the participant and reported by the participant. Questions were administered in isiZulu, the local language.

Exposure Assessment

HCT exposure is based on self-report collected annually in the HIV survey from 2006-2010. Specifically participants were asked annually: "Do you know your HIV status from previous HIV testing?" Participants who responded "no" to this question at their first visit and "yes" to this question at their second visit are considered "HCT-exposed" at baseline. Persons who responded "no" to this question at both their first and second visits are considered "HCT-unexposed" at baseline. Those who responded "yes" to this question at both time points were excluded, as we could not estimate when they sought HCT or what their covariate values would have been then. All participants who were HCT-exposed at baseline remained HCT-exposed for the remainder of follow-up. Some participants who were HCT-unexposed at baseline reported subsequent HCT-exposure (e.g., in their fourth survey round). For these participants, HCT was treated as a time-varying exposure. The first portion of person-time was characterized as HCT-exposed and the second portion was characterized as HCT-unexposed.

Outcome Assessment

The primary outcome in this analysis is HIV acquisition ascertained in the HIV survey. For participants who seroconverted, the time of HIV seroconversion was calculated as the midpoint between the last HIV-negative result and the first HIV-positive result minus thirty days. Thirty days were subtracted to allow for a window period between the time HIV was acquired and the time when it could have been detected [28]. For persons who did not acquire HIV during follow-up, their follow-up time extended from enrollment until their last HIV-negative or indeterminate test result minus thirty days. Persons who refused to participate in the sero-survey after their last HIV-negative test are characterized as "refusals" at the time of refusal.

Statistical Analyses

A directed acyclic graph was used to identify a minimally sufficient set of covariates to include in the analysis [29]. These included gender, age, year of study initiation, distance to the nearest study clinic, education, pregnancy status, fatherhood status, whether or not sexual debut had occurred, number of sex partners in the last year, and condom use at last sex [23, 30-32]. For a fraction of participants, information was missing for education, pregnancy, fatherhood, sexual debut, number of partners in the last year, and condom use at last sex. Multiple imputation using Markov Chain Monte Carlo simulation was used to impute missing values of these covariates separately for the HCT-exposed and HCT-unexposed participants [33]. Five complete imputed data sets were generated using PROC MI and results were combined using PROC MIANALYZE in SAS 9.2 [34].

Unadjusted HIV incidence rates were calculated in the two exposure groups and within strata of covariates (Table 5.2). Persons who were initially HCT-unexposed and became HCT-exposed contributed person-time to both exposure groups. We used Cox proportional hazard models to compare the association of HCT with time to HIV acquisition. We ran two main analyses: unadjustedand inverse probability of exposure and censoring weighted (i.e. a marginal structural model) (Table 5.3) [35-36]. Censoring weights were based on refusal. In all models, time since HCT was used as the primary analytic time scale [37] and the exposure and covariates were treated as time-varying. When time-varying confounders, such as sexual behavior, are influenced by a prior exposure, such as HCT, covariate-adjusted models can give biased results even in the absence of uncontrolled confounding [35]. In such settings, marginal structural models are more appropriate.

In these marginal structural models, inverse probability of exposure and censoring weights were constructed in a population with one observation per person-quarter [35]. Exposure weights were estimated as follows: the probability of each person receiving their exposure

history, conditional on their time-updated covariate history, was calculated using pooled logistic regression. We took the inverse of this probability as the denominator of the weight. The numerator of the weight was a similar probability, based only on time-fixed covariates. Together, these created a set of stabilized inverse probability of exposure weights. Stabilized inverse probability of censoring weights were estimated similarly, except that exposure history was entered into the model along with covariates. Exposure and censoring weights were multiplied to produce final stabilized weights. Weights were well-behaved with a mean of 1.02 (standard deviation=0.34) and a median of 0.98 (range =0.10-7.78). Values were attenuated at the 1st and 99th percentiles to avoid undue influence of extreme weights [38]. The hazard of HIV acquisition by HCT status was assessed in the weighted population using pooled logistic regression models to estimate discrete time hazard models with robust variance estimators and independent correlation matrices. All statistical analyses were implemented using SAS 9.2

Results

Study Population

Overall, 18,385 persons aged 15-24 years were enumerated in the catchment area in at least two surveillance rounds from 2006-2010 (Figure 5.1). Youth who reported knowing their HIV status at their first HCT report were excluded (N=3985, 22%), as were persons infected with HIV before baseline (N=1054, 6%). Persons with insufficient HCT information, HIV status information, or both and were also excluded. Most of these excluded persons were migrants who were enumerated but not residing in the catchment area for much of the time period (N=6013, 33%), and some (N=3372, 18%) were persons who declined participation. The study sample included the remaining youth (N=3959, 22%). Of these youth, 1167 (29%) were HCT-exposed and 2792 (71%) were HCT-unexposed at baseline. Of the persons who were HCT-unexposed, 38% became HCT-exposed during their follow-up period (N=1064) and the rest remained unexposed during follow-up (N=1728).

Population Characteristics

The study population was 51% male and 49% female with a median age of 17 (inter-quartile range: 16-20) (Table 5.1). Most women (83%) reported having never been pregnant and the most men (95%) reported never having fathered a child. At baseline, two thirds of participants reported not having experienced sexual debut (66%) and reported no sexual partners in the last year (68%), while 4% reported two or more partners in the last year. Of those who were sexually active, 43% reported condom use at last sex. Persons who were HCT-exposed at enrollment were more likely to be female, older, and more sexually experienced, with a greater proportion having experienced sexual debut, reporting $\geq$1 partner in the last year, and having been pregnant.

HIV Incidence and Time to Event Analysis

In the periods after HCT exposure, participants experienced 117 events over 3834 person-years of follow-up [incidence rate: 3.05 (95% CI: 2.55, 3.66) per 100 person-years]. In the periods of HCT non-exposure, participants experienced 131events over 4702 person-years of follow-up [incidence rate: 2.79 (95% CI: 2.35, 3.31) per 100 person-years] (Table 5.2).

In unadjusted Cox analysis, comparing HCT-exposed persons to HCT-unexposed persons, the hazard of HIV acquisition was 1.00 (95% CI: 0.78, 1.29) (Table 5.3, Figure 5.2). In the marginal structural model, the hazard of HIV acquisition was 0.59 (95% CI: 0.45, 0.78) (Table 5.3). Most of the change in estimate was attributed to weighting for gender, sexual debut, and having ever been pregnant. Results were not statistically different between men (HR: 0.66, 95% CI: 0.37, 1.38) and women (HR: 0.57, 95% CI: 0.41, 0. 78) or between those who were <20 years old (HR: 0.59, 95% CI: 0.43, 0.82) and those who were $\geq$20 years old (HR: 0.56, 95% CI: 0.31, 0.97).

Sensitivity Analyses

In a marginal structural model with inverse probability of exposure weights, but no censoring weights, the hazard ratio was essentially identical to the main effect: 0.59 (95% CI: 0.44, 0.78), suggesting that confounding, not censoring, was driving the difference between the unadjusted and weighted effects. In a marginal structural model without imputed covariates, the hazard ratio was similar, but estimated less precisely (HR: 0.64, 95% CI: 0.46, 0.89). Hazard ratios were similar in the first 1.5 years after HCT (HR: 0.65 (95% CI: 0.40, 1.05) as in the next three years after HCT (HR: 0.57, 95% CI: 0.40, 0.79), suggesting durability. In standard multivariate analysis adjusting for time-varying confounders, the hazard of HIV acquisition was 0.64 (95% CI: 0.48, 0.85).

Discussion

Our observations strongly suggest that youth living in this high prevalence environment experienced reduced HIV acquisition after HCT, an effect that was sustained for over four years. Youth who had received HCT and those who had not experienced a similar rate of HIV acquisition. However, youth who had sought HCT were at higher risk for HIV. They were more sexually experienced, making them more behaviorally vulnerable, and more likely to be female, making them more biologically vulnerable. Once these risk factors had been accounted for, it became clear that HCT had in fact been protective.

Our findings differ from similar studies conducted among adults, which suggested that HCT had no effect, or even a small harmful effect on HIV acquisition [14-15, 40]. Youth may be affected by HCT differently than adults. Socially, youth are less likely to be in marital or cohabitating unions than adults [19].HCT may be better able to facilitate adoption or continuation of condom use in the context of these non-cohabiting, non-marital unions [7]. Similarly, youth may be able to select other HIV-uninfected partners, an option that may not be possible in adults

in long-term unions. Additionally, youth are still in a formative period, and may be better able to adopt behavioral messages than adults who have already formed behavioral habits [18].

The causal mechanism underlying the relationship between HCT and HIV acquisition is not known. Exploratory work suggests that at later time points, youth who sought HCT were more likely to report condom use at last sex than youth who had not. Similar relationships were not seen for other behavioral factors, such as sexual debut or number of sex partners in the last year. HCT referral to medical male circumcision is unlikely to explain the effect, as it was not introduced into the catchment area until the very end of the study period at low volumes. Another possibility is that after HCT HIV-uninfected youth sought other partners who were also HIV-uninfected, something that is possible in an era with high HCT uptake.

Delivering HCT in non-stigmatizing ways is critical, as youth often report judgmental care-seeking environments [41]. Models of adolescent-oriented service delivery have been put forth, and understanding whether these models improve service uptake or sexual health outcomes is important [42]. The majority of youth reported their last HCT encounter was from a primary health center, with a substantial fraction reporting home-based or mobile testing. However, this question was only available at the end of the study, limiting our ability to assess the impact of testing modality.

Although HCT was protective in this population, youth who received HCT still experienced an extremely high HIV incidence rate of 3 per 100 person-years. Using HCT for delivery of or linkage to other prevention strategies is therefore essential. HCT may be more effective at changing behavior among HIV-uninfected adults in HIV-discordant relationships [43], and similar dyad-oriented strategies should be explored in youth in ongoing relationships. Additionally HCT must be used for linkage to medical male circumcision among HIV-uninfected young men [44] and pre-exposure prophylaxis among HIV-uninfected young men and women, when appropriate and available [45]. For HIV-infected persons, HCT has long been a means of

linkage to care for individual health, but is becoming even more important as treatment is used for prevention of onward transmission [46].

Large groups of youth were excluded or lost, which raises concerns about selection bias and non-generalizability. First, many persons who were HCT-exposed at the time of first report were excluded because we could not estimate when HCT exposure had occurred or what covariate values would have been at the time of exposure. If the rate of HIV acquisition had been higher in this group of testers, HCT would appear more protective than it truly was. Additionally, we excluded persons with insufficient HCT information, HIV information, or both. About two thirds (64%) of these persons were non-residents, typically persons residing outside of the catchment area for work. In contrast, only 4% of included youth were non-residents at baseline, suggesting our findings are best generalized to residents. Of the remaining excluded youth, many had sufficient HCT information (N= 1611), but insufficient HIV information. The proportion of these excluded youth that was HCT-exposed at baseline (25%) was comparable to the proportion of included youth that was HCT-exposed at baseline (29%), and covariate distributions by HCT status were similar as well. This suggests that outcome distributions may be comparable, as well, but this is not testable.

This analysis may not have fully accounted for all factors that influence the effect of HCT on HIV acquisition. Some sexual behaviors, such as sexual debut, may have been under-reported due to social desirability, a phenomenon that is likely as many HIV acquisition events (77/248) occurred among persons who had not experienced sexual debut. An alternative explanation for acquisition among youth who did not report debut is sexual abuse. In South Africa, boys 15-26 frequently report perpetrating rape [47] and school-age boys and girls report being victims of forced or coerced sex [48]. These factors may not have been captured in questions about debut and partnerships, but may have been important risk factors. Additionally, behavioral constructs, such as perceived risk, behavioral intentions, awareness of partners' HIV status and disclosure were not captured, and these factors could have affected results [49].

Given these potential sources of bias, replication of these results in similar settings is warranted. The ongoing study, Project Accept is assessing whether an intensive community-based HCT model is more effective at decreasing HIV acquisition than standard-of-care clinic based HCT [20]. Results will contribute critical new insights to this question.

HCT is a necessary first step for reducing HIV transmission through linkage of HIV-infected persons to treatment. Based on our observations, HCT may be effective at reducing HIV acquisition among youth, even in the absence of biomedical strategies. These findings are important in light of South Africa's recent debates about whether to implement HCT in secondary schools [13]. Although our analyses do not determine whether overall benefits of HCT outweigh the possible psychosocial harms among youth in this setting, HCT is likely beneficial from a prevention perspective. Promotion of HCT to South African youth may help slow the rate of HIV acquisition in this high risk population.

Tables and Figures

Table 5.1 Baseline Characteristics of the Study Population

	HCT-unexposed N=2792		HCT-exposed N=1167		Total N=3959	
Gender						
Female	1247	45%	690	59%	1937	49%
Male	1545	55%	477	41%	2022	51%
Age						
15-19	2268	81%	856	73%	3124	79%
20-24	524	19%	311	27%	835	21%
Year of study entry						
2006	1887	68%	609	52%	2501	63%
2007	553	20%	220	19%	773	20%
2008	309	11%	273	23%	582	15%
2009	43	2%	65	6%	108	3%
Distance to Nearest Clinic						
<2 km	838	30%	410	35%	1248	32%
2-5 km	1398	50%	559	48%	1957	49%
>5km	556	20%	199	17%	755	19%
Education						
≤primary	657	25%	206	19%	863	23%
some secondary	1801	68%	719	66%	2520	67%
≥ secondary	188	7%	170	16%	358	10%
Ever had sex						
Yes	728	29%	465	47%	1193	34%
No	1749	71%	532	53%	2281	66%
Condom Use at Last Sex (among sexually active)						
Yes	305	43%	192	42%	497	43%
No	407	57%	261	58%	668	57%
Sex Partners in Last 12 months*						
0	1799	73%	555	56%	2354	68%
1	595	24%	384	39%	979	28%
≥2	82	3%	46	5%	128	4%
Ever Pregnant (females)						
Yes	159	14%	146	24%	305	17%
No	984	86%	462	76%	1446	83%
Ever Father (males)						
Yes	51	4%	34	8%	85	5%
No	1341	96%	381	92%	1722	95%

Caption: Table 5.1 compares the baseline factors between the HCT-exposed and HCT-unexposed groups observed in this analysis. Covariate totals may not add up to the column total due to missing data.

Table 5.2 Incidence of HIV Acquisition by HCT Exposure and Covariates

Variable	Events	PYs	IRR	(95%	CI)
HCT					
Unexposed	131	4702	2.79	(2.35,	3.31)
Exposed	117	3834	3.05	(2.55,	3.66)
Gender					
Female	190	4344	4.37	(3.79,	5.04)
Male	58	4193	1.38	(1.07,	1.79)
Age					
15-19	188	6570	2.86	(2.48,	3.30)
20-24	60	1966	3.05	(2.37,	3.39)
Year of study entry					
2006	187	6120	3.06	(2.65,	3.53)
2007	42	1472	2.85	(2.11,	3.86)
2008	18	848	2.12	(1.34,	3.37)
2009	1	96	1.04	(0.15,	7.36)
Distance to Nearest Clinic					
<2 km	81	2804	2.89	(2.32,	3.59)
2-5 km	129	4166	3.10	(2.61,	3.68)
>5km	38	1567	2.43	(1.76,	3.33)
Education					
≤ primary	41	1724	2.38	(1.75,	3.23)
some secondary	177	5733	3.09	(2.66,	3.58)
≥ secondary	30	1080	2.78	(1.94,	3.97)
Ever had sex					
Yes	171	3730	4.58	(3.95,	5.32)
No	77	4806	1.60	(1.28,	2.00)
Condom use at last sex					
Yes	56	1397	3.08	(2.65,	5.21)
No	115	2333	4.93	(4.11,	5.92)
Sex Partners in Last 12 months					
0	84	4859	1.73	(1.40,	2.14)
1	152	3291	4.62	(3.94,	5.42)
≥2	12	387	3.10	(1.76,	5.46)
Ever Pregnant (females)					
Yes	78	1152	6.77	(5.42,	8.45)
No	112	3191	3.51	(2.92,	4.22)
Ever Father (males)					
Yes	6	221	2.71	(1.22,	6.04)
No	52	3972	1.31	(1.00,	1.72)

PY=person-year; IRR=incidence rate ratio; CI=confidence interval
Caption: Table 5.2 presents number of events, person years, incidence rate ratios, and confidence intervals by time-fixed and time-updated variables.

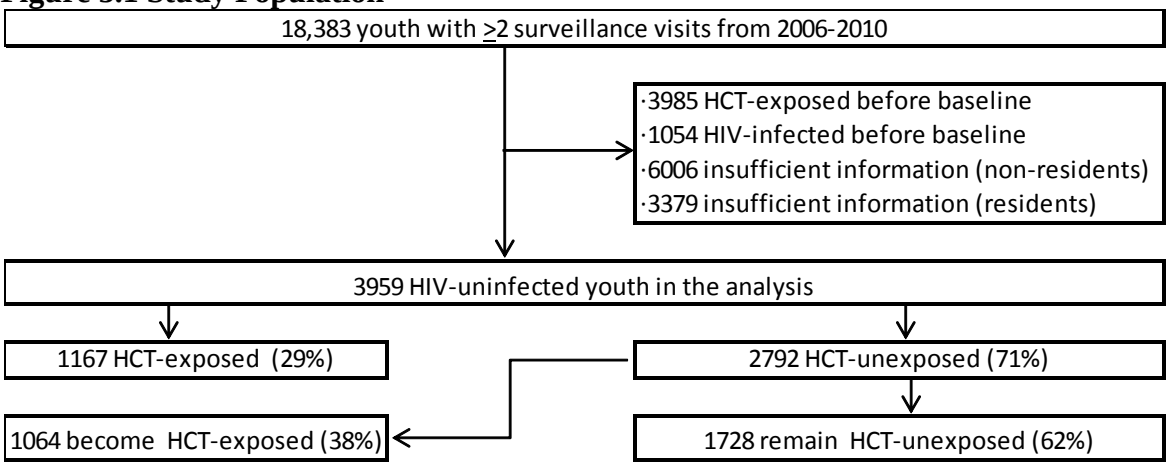
Table 5.3 Hazard of HIV Acquisition by HIV Counseling and Testing Status

	Unadjusted			MSM*		
	HR	(95%	CI)	HR	(95%	CI)
HCT						
unexposed	1.			1.		
exposed	1.00	(0.78,	1.29)	0.59	(0.45,	0.78)

HCT=HIV Counseling and Testing, HR=Hazard Ratio, CI=Confidence Interval, MSM=marginal structural model.

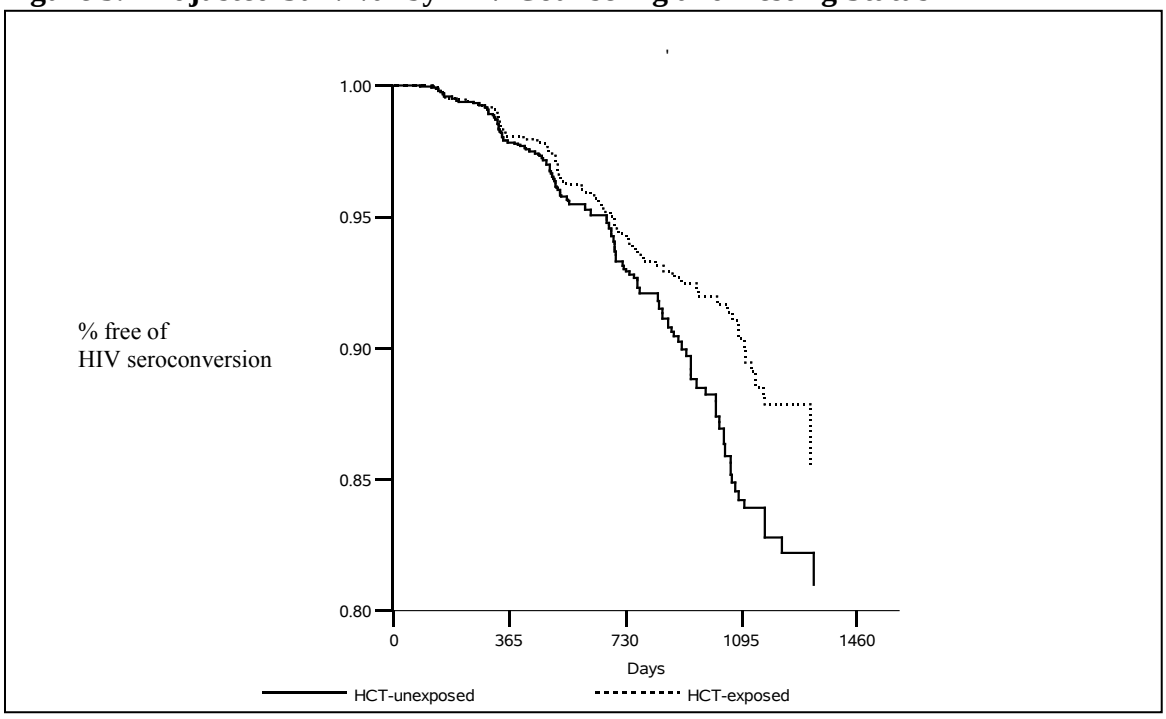
*Adjusted model controls for gender, age, distance to the clinic, year of study entry, educational attainment, sexual debut, number of sex partners in the last year, ever pregnant, ever fathered a child, and condom use at last sex. In the MSM, inverse probability of exposure and censoring weights are constructed using the same variables.

Figure 5.1 Study Population



Caption: Figure 5.1 illustrates the number of youth 15-24 in the catchment area with at least two surveillance visits from 2006-2010. It shows the proportion included in and excluded from the analysis, and the proportion who received HCT at baseline and over time.

Figure 5.2 Adjusted Survival by HIV Counseling and Testing Status



Caption: Figure 5.2 depicts the covariate-adjusted Kaplan Meier survival curves over a four year period comparing those who were HCT-unexposed to those who were HCT-exposed (HR=0.64).

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CHAPTER VI: Aim 3

The Awareness Framework:
A Novel Approach for Understanding HIV Testing and Disclosure in HIV-discordant Dyads

Introduction

HIV testing and counseling (HCT) is rapidly being brought to scale in sub-Saharan Africa [1]. Scale-up has been driven primarily by the goal of linking HIV-infected persons to treatment. But what impact will HCT scale-up have on HIV prevention, especially as new biomedical HIV prevention interventions are introduced?

Consistent with the *Couples HIV Testing and Counseling Guidelines* recently released by the World Health Organization, we assert that the prevention impact of HCT will likely hinge on whether both members of HIV-discordant dyads receive HCT and whether they share their HIV status with each other. However, we argue that a better understanding of the prevention impact of other HCT and disclosure strategies is needed. We introduce a novel framework for considering a person's awareness of his/her own HIV status (through HCT) and his/her partner's HIV status (through HIV disclosure) within HIV-discordant dyads. This framework is useful for understanding HCT trends, examining behavioral and biomedical risk in partnerships, and ultimately optimizing the impact of HIV prevention interventions.

Describing the Awareness Framework

HIV awareness within dyads involves two stages – testing and disclosure. In the first stage, persons can learn their own HIV status through HIV testing. In the second stage, they can inform their sex partners of their HIV status through disclosure. Within this framework, HIV

testing is a prerequisite for disclosure. In couples HCT, the two stages occur simultaneously in both partners.

For prevention purposes, these two stages of awareness must be considered within HIV-discordant dyads. HIV-discordant dyads account for *all* sexual transmission of HIV. We use the phrase "HIV-discordant dyad" broadly to refer to all sexual contacts with one HIV-infected and one HIV-uninfected person. These dyads can be homosexual or heterosexual, married or unmarried, long-standing or brief. We distinguish the term "HIV-discordant dyad," from the more commonly used terms HIV-discordant "couple" or "partnership" which typically refers to a subset of dyads who are in long-term, stable, and often marital or cohabiting relationships.

When HIV testing and disclosure in each member of the dyad are considered jointly, nine "awareness patterns" are possible (Table 6.1). In one extreme pattern, neither partner has been tested for HIV (pattern 1). In the other extreme pattern, both partners have been tested for HIV and have mutually disclosed their HIV status to each other (pattern 9). In an intermediate pattern, both partners have been tested individually but neither has disclosed to the other person (pattern 5). Six additional patterns reflect other combinations of testing and disclosure—two in which only one partner has been tested but has not disclosed (patterns 2 and 4), two in which one only partner has been tested and has disclosed (patterns 3 and 7), and two in which both partners have been tested, but only one has disclosed (patterns 6 and 8). Several patterns, such as neither partner having ever been tested (pattern 1), have been described in the literature. Other patterns, such as pattern 6 or 8, where both partners have been tested, but only one has disclosed, have rarely been considered.

Within an HIV-discordant dyad these patterns may change over time. For some dyads, mutual awareness (pattern 9) may occur soon after the relationship forms. For other dyads, it may take years to progress to mutual awareness. And for others still, especially those of brief duration, mutual awareness may never occur.

The Awareness Framework is likely to be an important mediator of HIV prevention, as depicted in Figure 6.1. Different HCT strategies, such as individual versus couple HCT or client-initiated versus provider-initiated HCT will likely result in different distributions of Awareness Framework patterns (i.e. pattern mixes). In turn, these pattern mixes are likely to affect the use of prevention strategies, including condoms, circumcision, pre-exposure prophylaxis (PrEP), and early antiretroviral therapy (ART) initiation, which reduce HIV transmission [2-3] or acquisition [3-8].

Relating HCT Strategies to the Awareness Framework

A first set of questions to consider with the Awareness Framework is how HCT strategies impact the HIV-discordant dyad pattern mix. Both the type and scale of HCT strategies are important.

The two main types of HCT strategies for individuals are voluntary counseling and testing (VCT) and provider initiated testing and counseling (PITC). For many years, the VCT paradigm accounted for most HCT in Africa. VCT is typically client-initiated and counseling-intensive. Recently, there has been a paradigm shift towards PITC with more limited counseling, but broader reach through opt-out methods [9-10]. This paradigm shift is likely to result in fewer pattern 1 dyads (where neither partner has been tested) and more pattern 2, 4 and 5 dyads (where at least one partner has been tested). However, due to less intensive counseling, PITC often results in lower rates of HIV status disclosure than VCT [11]. Consequently, a smaller proportion of dyads would progress from patterns 2, 4 and 5 (where at least one partner has tested) to patterns 3, 6, 8, and 9 (where at least one partner has disclosed).

Several HCT strategies are explicitly dyad-oriented, and these are likely to result in higher rates of disclosure. Couple's HCT has been implemented in stand-alone VCT settings [12], antenatal clinics [13], and home-based HCT [14], leading to dyads being mutually aware of each other's status (pattern 9). Intensive counselor-facilitated disclosure [15], provider-based partner

notification [16-17], and positive prevention counseling [18] have also yielded higher rates of disclosure by the HIV-infected partner (patterns 7, 8, and 9). Voucher-based recruitment, in which index patients are asked to give HCT vouchers to sex partners, also yields a higher share of dyads where at least one partner has disclosed (patterns 3, 6, 7, 8 and 9) [19]. Such strategies may be more costly and time-intensive in the short-term, but could prove worthwhile in the long-term if transmission events are averted.

The Awareness Framework Pattern Mix

A second, basic set of questions to address with the Awareness Framework is the prevalence of each pattern. Without this step it is not possible to determine which patterns are riskiest. Although all nine Awareness Framework patterns have never been characterized, individual self-report offers some insight into the pattern mix. In most African countries, before HIV treatment was available, very few persons had ever been tested, suggesting most dyads were in pattern 1 (neither partner tested) [20-23]. Currently, many individuals in SSA have been tested [1, 24] and in some settings a large proportion report disclosure to sex partners [25-29], suggesting a much broader distribution of patterns. However, even in a setting where 75% of persons have been tested and 75% of these persons have disclosed, only about a third of HIV-discordant dyads would be expected to have mutually tested and disclosed (pattern 9).[2]

Characterizing the pattern mix is possible in national or population-based surveys with questions on individual testing and disclosure, the ability to link dyads together, and HIV status. These characterizations would be an important first step to understanding the pattern mix at a point in time, and its evolution over time.

[2] If 75% of persons know their own HIV status and 75% of these persons disclose, then 56% of persons are likely to have tested and disclosed (0.75*0.75). The probability that both persons have tested and disclosed is 32% (0.56*0.56). This assumes testing and disclosure in the two partners are independent.

The Awareness Framework and HIV Prevention Strategies

A third application of the Awareness Framework is to assess how each pattern affects use of different HIV prevention strategies, including condoms, pre-exposure prophylaxis, circumcision or early ART initiation.

We hypothesize that pattern 9, where both partners have been tested and disclosed, is the most protective. In this pattern, both partners are aware that the HIV-uninfected partner is at risk for HIV acquisition. They can make decisions individually or together to protect the HIV-uninfected partner. The relative transmission risk within the other eight patterns is less straightforward. In each, the presence of risk within the dyad is uncertain for at least one partner. For example, for a pattern 5 dyad, even though both persons have been tested, neither has disclosed and therefore neither is aware that the dyad is HIV-discordant. Similarly, in patterns 3 or 7, when the status of one dyad member is known to both partners, they may assume incorrectly that the other dyad member has the same HIV status.

Condom Use

The combination of HCT and disclosure is known to have a strong impact on condom use. HIV-discordant dyads testing together (pattern 9), report dramatic increases in condom use, [31-32] and display lower HIV incidence rates [3, 33-34]. However, the association of each of the other eight patterns with consistent condom use is less clear because most studies have compared individuals' behavior in one *set* of patterns to individuals' behavior in a different *set* of patterns. For example, HIV-infected persons who know their own HIV status (patterns 4-9) tend to report much higher levels of condom use than HIV-infected persons who do not know their HIV status (patterns 1-3) [2, 16]. Such comparisons make it appear that all persons in patterns 4-9 have equal risk and that all persons in patterns 1-3 have equal risk, when in fact the risk within each set of patterns may vary considerably. Similarly, disclosure by HIV infected persons is often, though not always, associated with increases in condom use [25, 35-36], but this also has not been

studied by pattern. Separate comparisons of each pattern (1-8) to pattern 9 would provide a clearer picture of risk.

New Biomedical HIV Prevention Strategies

Access to new HIV prevention strategies will require HCT so persons can learn whether or not they are eligible. Only HIV-uninfected persons in patterns 2, 3, 5, 6, 8 or 9 (HIV-uninfected partner tested) will be able to access pre-exposure prophylaxis, or male circumcision. Similarly, only HIV-infected persons in patterns 4-9 (HIV-infected partner tested) will be able to access early ART initiation. Clearly, HCT scale-up is essential for biomedical prevention.

However, some of these patterns may be more strongly associated with adherence to biomedical prevention than others. Just as pattern 9 has been associated with higher adherence to condoms, it is likely to be associated with higher adherence to PreP and early ART initiation [39-40]. The groundbreaking HPTN 052 trial of early ART initiation by HIV-infected persons is an important example of this possibility [41]. This trial was conducted among mutually aware HIV-discordant dyads, all in pattern 9. In this trial not only did 95% of couples report consistent condom use, but adherence to ART was very high. When early ART initiation is implemented elsewhere, some persons will be in pattern 9 dyads, but others will undoubtedly be in pattern 4-8 dyads. HIV-infected persons in these other patterns may be less likely to use condoms and may face partner-level barriers to adherence. As a result, effectiveness might be undermined.

The Awareness Framework may also help explain different efficacy results in the PrEP trials. In both FEM-PrEP and Partners PrEP, participants were taking oral doses of FTC/TDF daily. But in the FEM-PrEP trial, HIV-uninfected women enrolled as individuals and could have been in any of several dyad patterns (2, 3, 5, 6, 8, or 9) or in HIV-concordant-negative dyads. In contrast, in the Partners PrEP study all participants were in mutually aware HIV-discordant dyads (pattern 9). In FEM-PrEP adherence was poor and PrEP was not efficacious [42]. In contrast, in Partners PrEP, adherence to PreP was excellent [43], and acquisition was reduced by 75% [8].

The different pattern mixes may have played a role in differences in adherence and ultimately efficacy.

Discussion

The Awareness Framework offers two novel contributions to our thinking about HIV prevention. First, it adds nuance to the discussion about HIV status awareness within HIV-discordant dyads. Although, many have advocated for couple-based strategies for Africa [44-48], none have delineated all of the possible alternatives to mutual awareness, even though they may be quite meaningful. Second, it helps us think about how different HCT strategies may translate into different utilization of many HIV prevention strategies and ultimately different effectiveness.

The Awareness Framework has real-world relevance for HTC programs. The types of HTC that are implemented will affect the Awareness Framework patterns which could have a profound impact on use of proven HIV prevention interventions. Assessing the shift in the Awareness Framework patterns over time will identify the gaps in testing or disclosure and in HIV-infected or HIV-uninfected persons. A better understanding of these gaps is necessary for determining how best to direct resources.

The Awareness Framework is a simple representation of the patterns of testing and disclosure within dyads. It is not a tool for understanding why people seek HCT or disclose their HIV status to their sexual partners, nor is it a conceptual model for understanding why some patterns are more protective than others. It also does not address whether all means of achieving a certain pattern are equally effective. Certainly the nine patterns are not the only factors underlying sexual risk-taking in partnerships. Gender, dyad stability, substance use, intimate partner violence, age gaps, and sexual communication are other key factors that are undoubtedly critical within dyads and should not be overlooked [35, 49-51].

Additionally, although HIV-discordant dyads are the primary unit of analysis within the framework, understanding how these dyads relate to sexual networks is important. HIV-

discordant dyads in riskier patterns will have a larger impact on HIV incidence if they have a central position in a sexual network or are engaging in higher levels of concurrency.

Additionally, although HIV-discordant dyads are the primary unit of analysis within the framework, understanding how these dyads relate to the sexual networks they are a part of is essential. HIV-discordant dyads in riskier patterns will have a larger impact on HIV incidence if they are engaging in higher levels of concurrency or have a more central position in a sexual network.

Overall the Awareness Framework provides a deeper, more nuanced consideration of HIV awareness and disclosure, which underlies all HIV prevention. Ignoring the complexity of testing and disclosure relationships within dyads may lead to oversimplified understandings of HIV prevention, suboptimal HCT strategies, and missed HIV prevention opportunities.

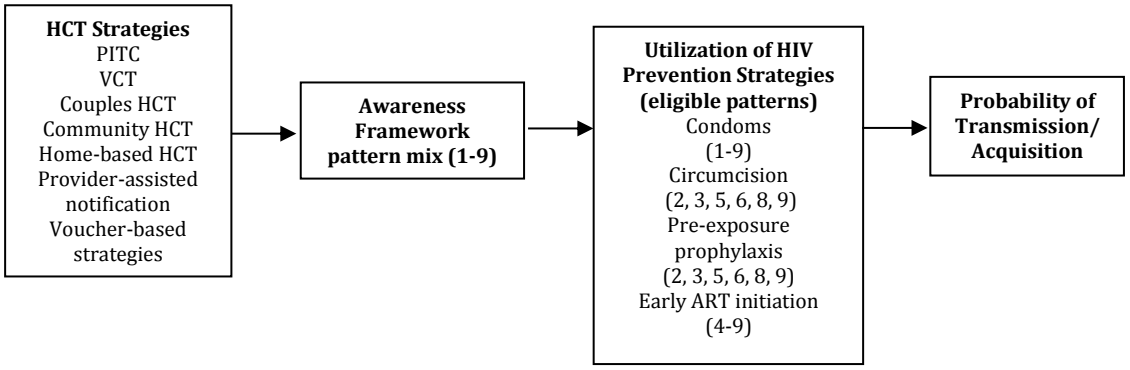
Tables and Figures

Table 6.1: The Awareness Framework—Nine Patterns for HIV-Discordant Dyads

	HIV-infected partner		
HIV-uninfected partner	**Has not tested for HIV, has not disclosed**	**Has tested for HIV, has not disclosed**	**Has tested for HIV, has disclosed**
Has not tested for HIV, has not disclosed	1 HIV-infected not tested, HIV-uninfected not tested	4 HIV-infected tested, HIV-uninfected not tested	7 HIV-infected disclosed, HIV-uninfected not tested
Has tested for HIV, has not disclosed	2 HIV-infected not tested, HIV-uninfected tested	5 HIV-infected tested, HIV-uninfected tested	8 HIV-infected disclosed, HIV-uninfected tested
Has tested for HIV, has disclosed	3 HIV-infected not tested, HIV-uninfected disclosed	6 HIV-infected tested, HIV-uninfected disclosed	9 HIV-infected disclosed, HIV-uninfected disclosed

Caption: There are nine possible awareness patterns within HIV-discordant dyads. The HIV-infected person may or may not have been tested, and if tested, may or may not have disclosed. Similarly, the HIV-uninfected person may or may not have been tested, and if tested, may or may not have disclosed. Understanding how each of the nine patterns is associated with uptake of and adherence to different HIV prevention strategies is important.

Figure 6.1: Role of the Awareness Framework in HIV Prevention



Caption: The Awareness Framework is likely to be an important mediator of HIV prevention. Different HCT strategies will lead to different Awareness Framework pattern mixes. In turn, these pattern mixes are likely to impact the utilization of different HIV prevention strategies, including condoms, circumcision, pre-exposure prophylaxis, and early ART initiation. These strategies have been shown to lower the probability of HIV transmission or acquisition.

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CHAPTER VII: Discussion

HIV Counseling and Testing (HCT) has been scaled up rapidly in many parts sub-Saharan Africa, over the last decade [1]. HCT is indisputably an essential first step for linking HIV-infected persons to care and treatment, but the impact of HCT on HIV prevention, especially among HIV-uninfected persons, has remained uncertain. This dissertation addressed the overarching question of what impact HCT has on HIV prevention. This overarching question can be broken down into several smaller questions. What impact does HCT have on behavioral and biomedical prevention strategies? What impact does HCT have on HIV acquisition versus HIV transmission? Does the impact vary in populations of individuals versus couples, men versus women, adults versus youth? Understanding the answers to some of these questions can inform the larger, more relevant public health question: how can the prevention impact of HCT be optimized? This dissertation addressed a subset of these questions in three aims: whether couples HCT had an impact on consistent condom use among HIV-discordant couples (Aim 1), whether HCT had an impact on HIV acquisition among HIV-uninfected youth (Aim 2) and whether nine different patterns of HCT and disclosure may impact prevention in HIV-discordant dyads (Aim 3).

Contributions

In this dissertation, two different types of HCT were shown to be protective in two distinct settings using two different outcome measures. In the first aim, couples' HCT was associated with high uptake of consistent condom use among HIV-discordant couples enrolled in an HIV prevention trial. Uptake occurred immediately and was sustained for one year of follow-

up. In the second aim, HCT was protective against HIV acquisition among HIV-uninfected youth over four years of follow-up.

In addition, we developed a new conceptual model, called the Awareness Framework, to help explain disparate findings in the HIV counseling and testing and disclosure literatures and facililate a more nuanced discussion of these topics.

Contributions of Aim 1

Aim 1 addressed whether HCT led to condom uptake and consistent condom use among HIV-discordant couples. I hypothesized that at baseline those recently tested would report more unprotected sex acts than those previously tested due to lack of awareness for part of the previous month. I also hypothesized that once both groups had been aware of their own HIV status and their partner's HIV status for the entire preceding month that sexual behavior would be comparable. My findings in this aim supported these hypotheses.

Three main comparisons were made in this aim. The first comparison was cross-sectional. At baseline, HIV-infected persons who had learned that they were HIV-infected $\leq$7 days previously were compared to persons who had known they were HIV-infected for >30 days. Those who had just learned that they were HIV-infected were much more likely to report engaging in unprotected sex (OR: 9.0) and having more unprotected sexual acts (relative number: 1.7) than persons who had been aware for >30 days. The second and third comparisons were longitudinal. In the second comparison, HIV-infected persons who had learned that they were HIV-infected $\leq$7 days before baseline were compared to themselves at later time points, when they had been aware that they were in HIV-discordant relationships for at least one month. These HIV-infected persons were considerably less likely to report any unprotected sex once they were aware that they were in an HIV-discordant relationship (OR: 0.03), a trend that persisted for one year (OR: 0.04). In the third comparison HIV-infected persons who had been aware of their own HIV status for >30 days before baseline, but not necessarily their partner's HIV status, were

compared to themselves one month later when they had known their own and their partners' HIV status for at least one month. Persons were somewhat less likely to report engaging in unprotected sex once they had been aware of both their own HIV status and their partners' HIV status for one month (OR: 0.45) and this also persisted for one year (OR: 0.66).

The strength of this analysis was that it included a comparison of two different groups at one point in time and comparisons of each group over time. If only the baseline comparison had been conducted, it would be possible to argue that a certain type of person (e.g. care-seekers), rather than HCT, explained baseline differences in condom use. Individual level factors could have confounded the relationship. If only the longitudinal comparisons had been conducted it would be possible to argue that other time-varying factors (e.g. participation in the study), and not HCT explained the uptake of consistent condom use. Temporal factors could have confounded the relationship. However, having both comparisons together makes it unlikely that *only* individual or *only* temporal factors were responsible for behavior change. If only individual level factors had been responsible, it would be difficult to explain the uptake of consistent condom use in *both* groups over time. If only temporal factors were responsible, it would be difficult to explain the cross-sectional differences at baseline.

It is worth noting that not only was mutual awareness of HIV status associated with consistent condom use, but it was *strongly* associated with consistent condom use. This relationship was not modified by gender or age. Additionally, after extensive exploration of the associations between demographic and dyad-level characteristics with consistent condom use, HCT was not simply *one* factor associated with consistent condom use, but rather appeared *the strongest* factor associated with consistent condom use.

Our results are consistent with previous findings suggesting that couples HCT leads to substantial increases in consistent condom use [2-5]. However, we addressed this question in a new context. This is the first study, to our knowledge, to rigorously assess the impact of couples' HCT on sexual behavior in South Africa, a country with a unique cultural and historical context,

including a long period of HIV denialism [6]. It was also the first study, to our knowledge, to address this set of questions in an era when HIV treatment was becoming more widely available. Different contexts could give rise to different findings. Replication of previous findings in new settings is a critical part of the scientific process. These findings add robustness to this body of knowledge.

Due to the measurement points of previous studies, most did not address the question of how quickly uptake of consistent condom use occurred following couples HCT. Understanding whether this is a behavior that couples adopt right away or one that takes time is important. Because Partners in Prevention HSV/HIV Transmission Study assessed sexual behavior one month after baseline, this question could be assessed in this analysis. Our analysis is one of the first to show that condom uptake occurred within the first week after couples HCT [9], suggesting one counseling session was sufficient for adoption of this behavior. This finding has important public health relevance for all HIV-infected persons, but particular importance within the context of acute HIV infection. During acute HIV infection persons are highly infectious for only a brief period. During this period, immediate adoption of consistent condom use could have large impacts on the onward spread of HIV [7]. Our findings merit replication in persons identified with acute HIV infection.

Contribution of Aim 2

Aim 2 addressed whether individual HCT led to lower rates of HIV acquisition among HIV-uninfected youth in a high prevalence part of South Africa. I had hypothesized that HIV acquisition rates would be higher in those who were aware of their HIV status from HCT. Results from this aim refuted this hypothesis. In unadjusted analysis, the hazard of HIV acquisition among those exposed- and unexposed- to HCT were not statistically different (approximately 3 cases per 100 person years). But after appropriate weighting for time-varying risk factors and censoring, HIV-uninfected youth who had received couples' HCT experienced a 30% lower

hazard of HIV acquisition than their HIV-uninfected counterparts. This effect did not attenuate over time.

The difference between the null unadjusted findings and the protective weighted findings is due to differences between testers and non-testers. Testers were at greater risk for HIV acquisition than non-testers. First, testers were more likely to be female, a group that is more biologically susceptible to HIV at young ages. They were more likely to have experienced sexual debut. And they were more likely to have ever been pregnant and sexually active in the last year. When I controlled for these factors in standard models or weighted for these factors in marginal structural models, it became apparent that HCT was in fact protective. HCT had made the high risk youth more like the low risk youth.

This is one of a few studies to address the impact of HCT on HIV acquisition longitudinally with an HIV endpoint [8-10]. There have been several studies to look at the associations between HCT and HIV status in cross-sectional data, but these studies cannot determine the temporal ordering of HCT and HIV acquisition. Similarly, there have been several longitudinal studies among HIV-uninfected persons assessing the impact of HCT on sexual behavior endpoints [11-12] or other biomarkers, such as gonorrhea or pregnancy [13]. However, to rigorously assess the question of HCT on HIV acquisition, HIV endpoints are needed. In this analysis, independence of the exposure and the outcome coupled with the large number of HIV endpoints allowed for this assessment.

This analysis focused on youth, a group at high risk for HIV acquisition and one in which the impact of HCT on HIV acquisition has not been assessed. Additionally, the ACDIS dataset was better able to address this question in youth. Most adults 25 years old and older had 1) already acquired HIV, 2) reported knowing their HIV status at the time of the first HIV report, 3) had migrated, or 3) refused to provide blood for the sero-survey. As a result, very few adults were eligible for inclusion in the analysis. For both scientific and practical reasons, this analysis was therefore restricted to youth.

Our findings differ from previous work conducted primarily among adults, suggesting HCT offered to individuals has no effect, or even a small harmful effect, on HIV acquisition [8-10]. The difference between our findings and previous findings may be due to differences in the analytic methods used or the populations under consideration.

Our analysis employed rigorous statistical methods. Other analyses used only baseline HCT status and baseline covariates, rather than time-varying HCT status and time-varying covariates. Our analysis was able to capture the time-varying nature of HCT, and appropriately account for time-varying factors. We did this using standard analytic methods and marginal structural models.

This was the first analysis to address this question exclusively among young persons. It is possible that young persons are able to change their behavior more easily than adults. In this area, due to the high price of lobola (bride price), marriage does not typically occur before the age of 25, and cohabitation before marriage is rare. Thus young persons in this cohort tend to be in less stable relationships, ones where condom negotiation may be easier. In exploratory analyses those who were HCT-exposed reported greater condom use at last sex than those who were HCT-unexposed in the period after HCT report, suggesting this may be the mechanism of action. Another possibility is that HIV-uninfected youth were more likely to find other HIV-uninfected sex partners. It is also possible that there is a cohort effect and that these young persons, growing up in a period when HIV is recognized and care is available, are more motivated to reduce their risk following HCT than persons in previous years. Whatever the reason, this is an especially timely issue as South Africa debates the introduction of HCT into secondary schools [14]. Replication of these results in experimental, quasi-experimental, or even other observational settings is important.

Contribution of Aim 3

Finally, in Aim 3 we developed a Framework to clarify thinking about the relationships between two types of HIV awareness in HIV-discordant dyads (testing and disclosure) and describe how these relationships may be associated with HIV prevention. The Framework can be used to enhance understanding of a variety of HIV prevention research, including Aims 1 and 2 of this dissertation.

Aim 1 contained three primary comparisons, as described above. These comparisons can also be described in terms of the Awareness Framework. Baseline results compared dyads in which the HIV-infected person had not tested (over until very recently) (patterns 1-3) with dyads in which the HIV-infected person had tested (patterns 4-9). Among HIV-infected persons, testing was more protective. The first longitudinal results compared dyads in which the HIV-infected person had not tested (patterns 1-3) with dyads in which both partners were mutually aware (patterns 9). Mutual awareness was much more protective than non-awareness. The second longitudinal results were a comparison of dyads in which at least the HIV-infected partner was aware of his/her own status (patterns 4-9) with dyads that are mutually aware (pattern 9). Mutual awareness was somewhat more protective than individual awareness by the HIV-infected partner.

It is more difficult to apply the Framework to Aim 2 because it is not possible to determine which youth were in HIV-discordant dyads. However, many youth, including all those who sero-converted, were in HIV-discordant dyads, unless they acquired HIV through a non-sexual route. Of these HIV-discordant dyads, among the HCT-unexposed youth, the HIV-uninfected partner had not tested (patterns 1, 4, or 7) and in the HCT-exposed youth, the HIV-uninfected partner had tested (patterns 2, 3, 5, 6, 8, or 9). Patterns in which the HIV-uninfected youth had tested were more protective than the patterns in which the HIV-uninfected youth had not tested.

In Table 7.1 I summarize the results from Aims 1 and 2 using the awareness framework patterns. When looking at these comparisons, there are a few points that are worth noting. First,

mutual awareness (pattern 9) is always in the more protective group. Second, non-awareness by both persons (pattern 1) is always in the less protective group. And third, all of the other patterns are sometimes in the more protective group and sometimes in the less protective group.

Although these sets of comparisons are informative, they are unsatisfying, because we are unable to isolate single patterns and instead are looking at associations between pattern mixes. For example, the baseline cross sectional comparison in Aim 1, compares HIV-infected persons who are aware (patterns 4-9) with HIV-infected persons who are unaware (patterns 1-3). Are all three HCT-unexposed patterns (patterns 1-3) equally risky? Are all six HCT-exposed patterns (patterns 4-9) equally protective? Or are certain patterns more prevalent and driving the associations? These questions cannot be answered with the available data, but are important areas for future research.

Summary of Contributions

Overall, Aim 1 adds to the robustness of the finding that learning that one is in an HIV-discordant couples is strongly associated with consistent condom use, and this is adopted soon after HCT. Aim 2 suggests that among youth HCT leads to a moderate reduction in HIV incidence. And Aim 3 helps explain some of the discrepancies in the literature and suggests a direction for future research.

Limitations

In spite of the contributions made in this dissertation, both analytic aims have important limitations. Both aims relied on secondary data analyses, and as such were constrained by information that was already available. These constraints raise concerns about both bias and generalizability. Aim 3 also has limitations related to what it can and cannot explain.

Aim 1 Limitations

The first potential source of bias in Aim 1 is information bias. Both the exposure and the outcome rely on behavioral self-report. Participants reported the timing of their first HIV test. They also reported on their own sexual behavior in the last month. Subjects may have had difficulty recalling this information and may have provided socially desirable responses. Importantly, these two sources of bias may have been non-independent [15]. There could be factors, such as poor memory or social desirability, that affected the reporting of both the exposure and the outcome. This bias could be differential. At baseline persons who were HCT-exposed may have been more likely to under-report unprotected sex than persons who were HCT-unexposed, as persons who knew they were HIV-infected may have experienced more social desirability. If this had occurred, baseline results would have been exaggerated. However, due to the large effect size seen at baseline, this phenomenon would have to be very strong to entirely nullify the findings.

It is also possible that there is residual confounding in Aim 1. Although a broad range of individual and dyad-level factors were explored as potential confounders, there may have been unmeasured factors that we could not control for. A personality trait, such as a person's predisposition to risk, is one possible unmeasured confounder. Risk-averse persons may have been more likely to seek HCT earlier and may have been more likely to use condoms, inducing a spurious association. However, if this were the case, the group who had been HCT-exposed earlier would be expected to not only report less unprotected sex at baseline, but also less unprotected sex over time. This was not the case. Persons who were HCT-exposed at baseline reported slightly more unprotected sex over time.

There is also a concern about who these results can be generalized to. Do stable couples who participate in the study differ substantially from stable couples eligible for the study who did not participate? Couples who participate in a prevention trial are more likely to be concerned

about prevention. By enrolling together they may also have more amicable relationships and be better able to negotiate prevention behaviors. If couples HCT were offered to all couples, would the same high rates of HCT be observed? This is something that cannot be judged from the data available from the trial.

A related concern is whether a study of stable HIV-discordant couples is important for the transmission dynamics of a generalized epidemic. Although risk may be high within HIV-discordant couples, some have argued that they are isolated from larger sexual networks. Some investigators have suggested that stable HIV-discordant couples in SSA may account for as little as 14% of transmission [16]. It is worth noting, however, that many couples in this trial were not disconnected from larger sexual networks. In spite of few couples reporting outside partners at baseline, the number of outside partners increased over time, and one third of transmissions seen in the overall study were not genetically linked to the HIV-infected partner, suggesting they came from an outside relationships [17]. These findings suggest that these dyads are, in fact, interconnected to other sexual networks, at least through the HIV-uninfected partners. Counseling for these HIV-discordant dyads must not only focus on the dyad, but also on how to negotiate sexual behavior outside the relationship.

Aim 2 Limitations

Although ACDIS theoretically has information on each variable for each person in each year, in reality there is considerable missing information. Many included participants had sufficient information for inclusion in the analysis, but missing exposure information, outcome information, or covariate information during follow-up. Additionally, many persons had insufficient information for inclusion in the analysis at all. I employed a number of analytic techniques to address these concerns—multiple imputation of missing covariates, inverse probability weighting of persons who refused participation at later time points, sensitivity analysis of longer gap-lengths between exposures, and exploration of persons who refused. In all

cases, results were robust to these assumptions and sensitivity analyeses. However, given the substantial amount of missing information, it is still possible that the relationship between HCT and HIV acquisition was different in the missing data than it was in the non-missing data.

Aim 3 Limitations

The main limitation of the Awareness Framework is that it does not capture the complexity of different contexts and sexual behavior. Certainly the nine patterns are not the only factors underlying sexual risk-taking in partnerships. Gender, dyad stability, substance use, intimate partner violence, age gaps, and sexual communication are other key factors that are undoubtedly critical within HIV-discordant dyads and should not be overlooked [18-21]. However, although the awareness framework does not address these factors explicitly, the relationship between the Awareness Framework and these other factors of interest can be accommodated easily. For example, riskiness of the nine patterns can be assessed separately among men and women.

The framework may be also be adding unnecessary complexity. Once the framework is properly studied, it may turn out that certain patterns do not exist or do not differ from one another. For example, it may be incredibly rare for an HIV-infected person to have tested and disclosed, but for an HIV-uninfected person to have tested, but *not* disclosed (pattern 8). Further exploration could also reveal that certain patterns are equally risky, such as when an HIV-uninfected person has tested, but not disclosed (pattern 2) and when an HIV-uninfected person has tested and disclosed (pattern 3). However, without future research this cannot be determined.

Future Research

Research Stemming from Aim 1

One critical question that Aim 1 could not address was whether it was mutual awareness of each other's HIV status, the nature of couples counseling, or both together that was protective.

Answering this question would require comparisons of different types of mutually aware dyads (both in pattern 9). Assessing this question would require a comparison of couples who had undergone couples HCT together with couples who had undergone HCT separately and then disclosed to one another. Such a comparison would provide insight into whether it was the couples counseling or simply the mutual awareness that was the more protective factor.

Research Stemming from Aim 2

Findings from Aim 2 suggest several directions for future research. First, given the potential for bias in this analysis or differences between populations, replication of these results in similar datasets is important. There are other sites throughout sub-Saharan Africa that collect similar demographic and HIV surveillance data and replication at these sites is warranted [22]. Second, although we conducted exploratory analyses of the underlying causal mechanisms, a more detailed assessment of the causal mechanisms is important. There could be factors that were not assessed, such as sero-sorting, that were important. And third, although there has been rapid expansion of HCT in the catchment area since 2004, there are still many youth (both HIV-infected and HIV-uninfected) who do not know their HIV status [23]. Research is needed to compare the effectiveness and cost-effectiveness of different strategies, such as clinic-, home-, and school-based testing for HCT uptake among youth.

Research Stemming from Aim 3

The Awareness Framework suggests three key directions for future research. First, work is needed to simply asses the pattern mix in different populations. Second, a better understanding of which HCT strategies lead to each pattern mix is needed. And third, there is a need for a better assessment of how each pattern is associated with uptake of and adherence to different behavioral and biomedical HIV prevention strategies, such as circumcision [24-26], pre-exposure

prophylaxis, [27-29], and early treatment initiation [30]. Findings from this research will inform discussions about how best to use HCT to maximize a range of HIV prevention interventions.

Conclusions

HCT has been introduced rapidly and intensively into the South Africa context. Nonethless, current HCT strategies rarely involve partners and frequently do not reach youth. The next phase of HCT policy must address HIV prevention more aggressively, both in terms of the populations targeted and the types of services that are delivered. For HIV infected persons this will of course mean addressing the landmark findings that early ART intiatition is effective at reducing transmission. But it must also involve using HCT to identify HIV-discordant dyads and ensure that they are mutually aware, and reaching youth with HCT before they become infected. South Africa's HCT campaign was a successful step towards better coverage, but it cannot be the final step. The prevention challenges that South Africa faces are too great.

Tables and Figures

Table 7.1: Comparisons of Aims 1 and 2 using the Awareness Framework Patterns

	Reference Group	Comparison Group	Outcome
Aim 1			
Cross-sectional	1, 2, 3	4, 5, 6, 7, 8, 9	4-9 are more protective.
Longitudinal 1	1, 2, 3	9	9 is more protective
Longitudinal 2	4, 5, 6, 7, 8	9	9 is more protective
Aim 2			
Longitudinal 2	1, 4, 7	2, 3, 5, 6, 8, 9	2, 3, 5, 6, 8, and 9 are more protective

Caption: Table 7.1 summarizes the comparisons made in this dissertation using the awareness framework. In this dissertation, pattern 9 is always in the more protective group and pattern 1 is always in the less protective group. The other patterns are sometimes more protective and sometimes less protective.

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