

Antiretroviral Treatment as Prevention in African HIV-1 Serodiscordant Couples:
Understanding the Challenges and Opportunities

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Abstract

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Epidemiology

The studies described in this dissertation examine the relationship between use of antiretroviral therapy (ART) and biologic and behavioral factors related to HIV-1 transmission risk in stable heterosexual HIV-1 serodiscordant African couples. ART is recommended for all HIV-1 infected persons, regardless of CD4 count, to reduce HIV-1 related morbidity, mortality and risk of transmission to uninfected partners. ART is a cornerstone of combination HIV-1 prevention, and optimizing use of ART, both for treatment and prevention, is an urgent public health priority.

The complementary prospective studies presented in this dissertation were secondary analyses of data from the Partners PrEP Study, a randomized clinical trial of daily oral pre-exposure prophylaxis (PrEP) to decrease HIV-1 acquisition among HIV-1 uninfected members of serodiscordant couples in Kenya and Uganda. This work includes prospective studies of: 1) correlates of failure to achieve plasma viral suppression and virologic rebound after initial suppression, 2) frequency, magnitude and correlates of seminal HIV-1 RNA shedding in men initiating ART, 3) residual HIV-1 transmission risk during the first 6 months of ART, and 4) sexual risk behavior before and after ART.

Younger age was associated with delayed ART initiation, failure to achieve viral suppression, and increased risk of virologic rebound after initial suppression. Seminal HIV-1 RNA shedding was infrequent

and present at low levels in HIV-1 infected African men with suppressed blood HIV-1 RNA. There were no HIV-1 transmission events on suppressive ART. We observed residual HIV-1 transmission risk during the first 6-months of ART, prior to complete viral suppression in blood and genital secretions. Importantly, substantial risk compensation did not occur following ART initiation among HIV-1 infected persons with known uninfected partners.

Results from this dissertation contribute further evidence of the effectiveness of ART for HIV-1 prevention, and provide reassurance that HIV-1 transmission risk declines after starting ART. As treatment guidelines evolve from *wait-and-treat* to *test-and-treat*, scaling up access to HIV-1 testing, improving linkage and retention in care, and achieving high ART coverage and complete viral suppression at individual and population levels are essential to achieving zero new HIV-1 infections and zero AIDS-related deaths.

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DEDICATION

To my great grandmother Kezia Babisherekamu (1879-1993), grandmother Huldah Kasabiiti Nyakatukura (1912-2013), and mother Lucy Nyangoma Karani (1935-2003) for a Godly heritage.

To Perpetua Gihanga Karani (1944-2015).

To my family, Lindi, Lucy, and Lisa whose love and support keep me going.

To my father Peter Karani,

and my siblings Valerie, Linnet, Sarah and Simon,

I am because you are.

Chapter 1. Introduction

After >30 years of the HIV-1 epidemic, where there were an estimated 1.5 million new HIV-1 infections in 2013 [1]. Demographic and Health Survey (DHS) data from 20 sub-Saharan African countries indicate that 29% (range, 10-52%) of new HIV-1 infections occur within stable heterosexual HIV-1 serodiscordant couples, in which one of the partners is infected with HIV-1 and the other is not [2]. Many factors could contribute to the high risk of HIV-1 transmission in these couples, including perception of low risk among heterosexual couples that have lived together for years, condom fatigue, and fertility desire, all of which would lead to unprotected sex, and risk of HIV-1 transmission. Thus, serodiscordant couples are a priority population for novel, effective HIV-1 prevention strategies, given their high risk for HIV-1 transmission, risk of AIDS orphans, and small numbers relative to the general population.

Antiretroviral therapy (ART) has personal health benefits (decreased HIV-1 morbidity and mortality) and public health benefits (prevention of HIV-1 transmission), and is recommended by the World Health Organization for all persons following an HIV diagnosis [3]. The public health benefits of ART include protection from HIV-1 infection for uninfected sexual partners, and future children during pregnancy and breastfeeding. Antiretroviral-based HIV-1 prevention strategies, including ART to reduce the infectiousness of HIV-1 infected persons, are among the most promising new approaches for dramatically decreasing HIV-1 spread. ART is a cornerstone of combination HIV-1 prevention, and increasing ART uptake is an urgent public health priority. This dissertation addresses key questions about the effectiveness of ART for HIV-1 prevention among African serodiscordant couples. Here, we provide a brief overview of each research question.

ART, viral suppression and HIV-1 transmission risk

ART substantially decreases the risk of sexual HIV-1 transmission to uninfected partners, by decreasing plasma and genital HIV-1 RNA to undetectable levels within six months of treatment initiation in the majority of HIV-1 infected persons [4, 5]. HIV-1 RNA concentrations in plasma and the genital tract are the prime determinant of HIV-1 transmission risk. Each log increment in plasma HIV-1 RNA

concentrations is associated with a 2.45-fold (95% CI: 1.85, 3.26) increase in risk of sexual transmission of HIV-1 [6]. HIV-1 RNA concentrations in seminal and endocervical secretions independently predict HIV-1 transmission risk after controlling for plasma HIV-1 RNA quantity: each log increment in genital HIV-1 RNA is associated with a 2.20 fold increase in female-to-male transmission risk and a 1.79 fold increase in male-to-female transmission risk [7].

Proof of concept is also provided by vertical HIV-1 transmission studies in which there is a positive direct correlation between maternal plasma HIV-1 RNA and risk of HIV-1 transmission to the infant. In a collaboration of 7 prospective studies from Europe and the United States, HIV-1 transmission rates before, during, and after delivery were 1% for women with undetectable plasma HIV-1 RNA quantity (<400 copies/mL) compared with 23% for women with HIV-1 RNA >30,000 copies/mL [8]. Among 11,515 HIV-1 infected pregnant women in the United Kingdom and Ireland, vertical transmission rates were correlated with lower maternal plasma HIV-1 RNA concentrations: 0.09, 1.0 and 2.6% transmission rates for viral loads <50, 50-399 and 400-999 copies/mL, respectively [9]. The public health impact of high ART coverage has been demonstrated by population-level reductions in HIV-1 incidence in British Columbia (52%) [10], and South Africa (38%) [11]. The strong dose-response relationship between plasma and genital HIV-1 RNA quantity and HIV-1 transmission risk, and the reduction in HIV-1 infectiousness following ART start, provide biologic plausibility for the decrease in HIV-1 transmission risk reported in a randomized clinical trial and nine observational studies of serodiscordant couples [4, 12-20]. These data demonstrate the substantial public health potential of ART for HIV-1 prevention. Understanding the prevalence and correlates of plasma viral suppression will be important to help identify preventable factors related to viral non-suppression, and evaluate residual HIV-1 transmission risk prior to complete viral suppression.

ART and seminal HIV-1 shedding

Although ART effectively suppresses viral replication in plasma and the genital tract [21], several studies have shown that intermittent genital HIV-1 RNA shedding (IHS) occurs in approximately 10-40% men on ART, despite suppressed plasma HIV-1 RNA concentrations and lack of detectable sexually transmitted

infections [22-24]. The cause of IHS is not known. HIV-1 RNA persistence in semen could be due to incomplete ART penetration [25], HIV-1 compartmentalization (distinct viral genotypes in blood and genital tract) [26], or genital inflammation [27]. HIV-1 RNA concentrations during IHS are greater in men than women [28], and their clinical importance is not fully understood. IHS is thought to be distinct from intermittent plasma HIV-1 RNA levels ('blips'), which are thought to result from random biological and statistical variation around a mean HIV-1 RNA concentration [29]. Intensified ART with raltegravir and maraviroc, medications with high semen penetration, reduces but does not completely eliminate IHS during the first 3 years of effective ART [30]. Thus, some individuals may continue to be infectious to their sexual partners when on plasma suppressive ART. In one study, IHS in the presence of suppressed plasma HIV-1 RNA resulted in HIV-1 transmission in men who have sex with men (MSM) [31]. Whether IHS results in heterosexual HIV-1 transmission is unknown. Better understanding of IHS in the presence of effective ART will help inform the durability of ART for HIV-1 prevention for serodiscordant couples [32].

ART and sexual risk behavior

Risk compensation occurs when individuals who feel protected from a health risk engage in risk behaviors because of changes in risk perception [33]. Combination HIV-1 prevention interventions including condoms, male circumcision, topical microbicides, pre-exposure prophylaxis (PrEP), and ART could have the unintended consequence of increasing sexual risk behavior because of improved physical health and life expectancy, decreased perception of HIV-1 risk [34], and safe sex fatigue in stable partnerships. Mathematical models have suggested that the preventive benefits of expanded ART access could be offset by increased sexual risk behavior [35-37], perhaps because ART use modifies risk perception when HIV-1 is no longer considered to be a life-threatening disease [38]. In addition, ART prolongs survival potentially increasing duration of infectiousness, and lifetime risk of HIV-1 transmission. As ART availability increases in sub-Saharan Africa, attitudes about HIV-1 may be changing, with increased sexual activity following ART, coupled with increases in the proportion reporting multiple partners and unprotected sex with serodiscordant or causal partners. Better understanding of the effect of ART on sexual risk behavior in known serodiscordant couples in sub-Saharan Africa is needed.

This dissertation provides information on the preventive effectiveness of ART for HIV-1 transmission. We

leveraged high-quality data from a randomized PrEP trial in a large cohort of African HIV-1 serodiscordant couples, in which HIV-1 infected partners initiated ART, to evaluate three specific aims regarding ART use and viral suppression, HIV-1 transmission risk, and changes in sexual risk behavior. The first aim describes the frequency of achieving viral suppression, and correlates of suppression in blood and semen. The second aim evaluates the residual risk of HIV-1 transmission during the first six months of ART use when viral suppression in plasma and genital secretions may be incomplete. The third aim tests whether ART use is associated with increased frequency of high-risk sexual behavior. The data presented in the following chapters underscore the prevention benefits of HIV-1 treatment, highlight the risk of HIV-1 transmission soon after ART initiation and reassure that risky sexual behavior does not immediately occur after starting ART.

Chapter 2. Younger age predicts failure to achieve viral suppression and virologic rebound among HIV-1 infected persons in serodiscordant partnerships

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Younger Age Predicts Failure to Achieve Viral Suppression and Virologic Rebound among HIV-1 Infected Persons in Serodiscordant Partnerships

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ABSTRACT

Background: Antiretroviral therapy (ART) markedly reduces the risk of HIV-1 transmission in serodiscordant partnerships. We previously found that younger age and higher CD4 counts were associated with delayed initiation of ART by HIV-1 infected partners in serodiscordant partnerships. Among those initiating ART, we sought to explore whether those same factors were associated with failure to achieve viral suppression.

Methods: In a prospective study of HIV-1 infected persons who had a known heterosexual HIV-1 uninfected partner in Kenya and Uganda (Partners PrEP Study), we used Cox proportional hazards regression to evaluate correlates of viral non-suppression (HIV-1 RNA >80 copies/mL).

Results: Of 1035 HIV-1 infected participants initiating ART, 867 (84%) achieved viral suppression: 77% by 6 months and 86% by 12 months. Younger age (adjusted hazard ratio [aHR] 1.05 for every 5 years younger; $p=0.006$), lower pre-treatment CD4 count (aHR 1.26; $p=0.009$ for ≤ 250 compared with >250 cells/ μL) and higher pre-treatment HIV-1 RNA quantity (aHR 1.21 per \log_{10} ; $p<0.001$) independently predicted failure to achieve viral suppression. Following initial viral suppression, 8.8% (76/867) experienced virologic rebound (HIV-1 RNA >200 c/mL): 6.3% and 11.5% by 6 and 12 months after initial suppression, respectively. Age was the only factor associated with increased risk of virologic rebound (aHR 1.33 for every 5 years younger; $p=0.005$).

Conclusions: For HIV-1 infected persons in serodiscordant couples, younger age was associated with delayed ART initiation, failure to achieve viral suppression, and increased risk of virologic rebound. Motivating ART initiation and early adherence is key to achieving and sustaining viral suppression.

Introduction

There were an estimated 1.5 million new HIV-1 infections in sub-Saharan Africa in 2013, most of which were sexually transmitted [39]. The major determinant of sexual HIV-1 transmission risk is the concentration of HIV-1 RNA in plasma and genital secretions [6, 7], and antiretroviral therapy (ART), which suppresses systemic and genital HIV-1 replication, decreases sexual HIV-1 transmission by >90% [4, 40]. The World Health Organization (WHO) recommends ART initiation regardless of CD4 count for HIV-1 infected persons with uninfected partners to prevent sexual transmission of HIV-1 [41]. Achieving and sustaining viral load suppression is a key outcome of the HIV-1 treatment continuum and is critical to the success of ART for HIV-1 prevention.

The concentration of HIV-1 RNA in plasma is the principal indicator of ART prevention effectiveness. Nonadherence to ART increases the likelihood of poor virologic outcomes, including viral non-suppression, virologic rebound, disease progression, and HIV-1 transmission, including transmission of drug-resistant virus [42, 43]. In resource-rich settings, younger age predicts nonadherence, viral non-suppression, virologic rebound and loss to follow up [44]. Correlates of ART non-adherence include lower income, depression, alcohol and substance use, and lack of social support [45-49]. Nonadherence also occurs when asymptomatic individuals on ART skip doses or stop treatment completely because they feel well [50]. As ART for HIV-1 prevention is scaled up, and ART is prescribed to healthy individuals with higher CD4 counts more generally, it will be critical to understand and address preventable factors related to viral non-suppression.

We previously reported that younger individuals and persons with higher CD4 counts in HIV-1 serodiscordant partnerships in Kenya and Uganda were more likely to delay ART initiation [51]. Here, we evaluated correlates of non-suppression among the subset of HIV-1 infected partners who initiated ART. We sought to evaluate whether factors that predicted delay in ART initiation were similarly related to failure to achieve viral suppression and virologic rebound after initial suppression.

Materials and Methods

Study cohort and procedures

We conducted a prospective study among HIV-1 infected partners in HIV-1 serodiscordant couples enrolled in the Partners PrEP Study, a randomized clinical trial of daily oral pre-exposure prophylaxis (PrEP) to decrease HIV-1 acquisition among HIV-1 uninfected members of serodiscordant couples (ClinicalTrials.gov NCT00557245) [52]. Between 2008 and 2012, 4747 heterosexual HIV-1 serodiscordant couples were followed at nine research sites in Kenya and Uganda. HIV-1 uninfected partners were randomly assigned to daily oral PrEP or placebo, and followed monthly for up to 36 months. At the time of enrollment, HIV-1 infected partners were not eligible for ART according to Kenya and Uganda national guidelines, which early in the study recommended ART only for those with symptomatic HIV-1 disease or CD4 counts <250 cells/ μ L and were subsequently revised to CD4 counts \leq 350 cells/ μ L in July 2010 (Kenya) and April 2012 (Uganda). HIV-1 infected participants were followed quarterly and received regular clinical monitoring. Those who became eligible during follow-up for ART were referred to partnering HIV-1 care programs. ART and adherence counseling were provided by referral partners or at on-site clinics affiliated to the site organization. All participants received HIV-1 prevention services including regular individual and couple HIV-1 counseling, condoms, risk reduction counseling, plus syndromic or etiologic diagnosis and treatment for sexually transmitted infections (STIs).

CD4 testing was done at baseline and every six months thereafter at study site laboratories using BD FACSCalibur or BD FACSCount instrumentation (BD Biosciences) [53]. Plasma for HIV-1 RNA quantification was collected at enrollment and every six months thereafter, and archived and batch tested at the University of Washington after the end of the study using the Abbott m2000 Real-Time HIV-1 RNA assay (Abbott); the lower limit of quantification was 80 copies/mL. Viral load testing was not standard of care, and results were not available until after the study ended.

Statistical analysis

Participants were included in the analysis if they initiated ART during study follow-up, had a pre-ART plasma HIV-1 RNA concentration >80 copies/mL, and had at least one HIV-1 RNA quantification after starting treatment. Failure to achieve viral suppression (the primary outcome) was defined as plasma HIV-1 RNA concentration >80 copies/mL. We used a Cox proportional hazards regression model to assess

factors related to viral non-suppression. Baseline factors evaluated included age, gender, years of education, duration of partnership and known HIV-1 serodiscordant status, monthly income, alcohol use, pre-treatment CD4 count and HIV-1 RNA concentration. Unprotected sex in the prior month and pregnancy were evaluated as time-varying covariates. Factors with p-values ≤ 0.20 in bivariate analyses were included in multivariate models. In sensitivity analyses, we repeated our primary analysis by including subjects who achieved viral suppression prior to reporting ART use (although this potentially indicated unreported ART use prior to first reporting having started ART [54]) and by using a different cutoff (HIV-1 RNA concentration >400 copies/mL) to define viral non-suppression. In addition, we assessed factors associated with virologic rebound, which we defined as HIV-1 RNA concentration >200 copies/mL following initial viral suppression. Statistical analyses were performed using SAS version 9.3 and Stata 12.1.

Ethical approval

The Partners PrEP Study was approved by the University of Washington Human Subjects Review Committee and ethics review committees at all collaborating institutions. All participants provided written informed consent.

Results

Participant characteristics

Of the 4747 HIV-1-infected participants enrolled and followed in the Partners PrEP Study, 1817 initiated ART during study follow-up, of whom 1035 (57%) were included in the primary analysis (Figure 1). The median age was 35 years (interquartile range [IQR] 28-41), and 560 (54%) were women (Table 1). Most (98%) were married or cohabiting with their HIV-1 uninfected partner. The median duration of partnership was 9 years (IQR 3-16), and 263 (26%) reported having unprotected sex with the study partner at enrollment. The median body mass index was 22 kg/m^2 (IQR 20-24) for women and 21 kg/m^2 (IQR 20-23) for men. Women had higher median pre-ART CD4 counts than men (260 vs. 246 cells/ μL ; $p < 0.0001$). The median HIV-1 RNA plasma concentration prior to ART start was $4.44 \log_{10}$ copies/mL (IQR 3.90-4.89): 4.57 and $4.30 \log_{10}$ copies/mL in men and women, respectively. As previously reported, study retention

was high in the Partners PrEP Study, with at least 91% of HIV-1 infected partners retained at all study visits [55].

Plasma viral suppression and correlates of non-suppression

After ART initiation, HIV-1 infected participants were followed for 467 person-years for the assessment of viral suppression, with a median duration of follow-up of 13.8 months (IQR 7.8-19.4). Overall, 867 (84%) achieved viral suppression and the median time to first viral suppression was 3.1 months (IQR 2.8-5.6). The cumulative probabilities of achieving viral suppression at 3, 6, 12 and 24 months after starting ART were 46.7%, 76.7%, 86.0% and 90.1%, respectively (Figure 2).

In bivariate analyses, younger age ($p=0.01$), lower CD4 count prior to treatment initiation ($p=0.003$) and higher pre-treatment HIV-1 RNA concentration ($p<0.001$) were significantly associated with viral non-suppression (Table 1). In the adjusted model, the likelihood of viral non-suppression increased by 5% for every 5-year decrease in age (adjusted hazard ratio [aHR] 1.05; $p<0.006$). Lower pre-treatment CD4 count (aHR 1.26 for ≤ 250 compared with >250 cells/ μL ; $p=0.009$) and higher pre-treatment HIV-1 RNA concentrations (aHR 1.21 per \log_{10} ; $p<0.001$) were also significantly associated with failure to achieve viral suppression in plasma.

In sensitivity analyses, with viral non-suppression defined as HIV-1 RNA >400 copies/mL, our findings were similar to the primary analysis (Table 2). Failure to achieve viral suppression was independently associated with younger age ($p=0.01$), lower pre-treatment CD4 count ($p=0.004$) and higher pre-treatment HIV-1 RNA quantity ($p=0.008$). Similar findings were obtained when HIV-1 infected persons who achieved viral suppression prior to reporting ART use [$N=557$, Figure 1] were included (Table 2).

Virologic rebound

The 867 HIV-1 infected partners who achieved viral suppression were followed for 688 person-years for assessment of virologic rebound. Of the 76 persons (8.8%) who experienced virologic rebound (HIV-1 RNA >200 copies/mL), 51 (67%) were women. The cumulative probabilities of virologic rebound at 6 and 12 months after viral suppression were 6.3% and 11.5% respectively (Figure 3). The median HIV-1 RNA

concentration at the first occurrence of virologic rebound was 1244 copies/mL (IQR 208-7894); however, 24% (18/76) subsequently were suppressed (HIV-1 RNA<80 copies/mL) prior to their last study visit. In bivariate analyses, age (HR 1.35 for every 5 years younger; 95% CI: 1.18-1.54; p<0.001) and female gender (HR 1.95; 95% CI: 1.20-3.16; p=0.007) were associated with virologic rebound, but pregnancy (p=0.31) and pre-treatment HIV-1 RNA concentrations (p=0.67) were not. After adjusting for gender, age remained the only independent predictor of virologic rebound (aHR 1.33 for every 5 years younger; 95% CI: 1.14-1.56; p=0.005); gender was not statistically significant after controlling for age (p=0.8).

Discussion

In this prospective study of approximately 1000 East African HIV-1 infected persons in serodiscordant partnerships, nearly all HIV-1 infected persons achieved plasma viral suppression after starting ART. Younger age, lower CD4 count and higher HIV-1 RNA concentrations at treatment initiation independently predicted failure to achieve viral suppression in plasma. Younger age was the only significant factor associated with virologic rebound after initial viral suppression. In previous work from this cohort, younger age and higher pretreatment CD4 counts predicted delays in ART initiation in this same population. Unique to this population, all individuals initiating ART had a known HIV-1 uninfected partner, and all were counseled about both treatment and prevention benefits of ART.

Younger age has been associated with lower rates of HIV-1 virologic suppression in prior studies. A review of 49,921 HIV-1 infected persons on ART in 33 European cohorts found that increasing age correlated with better virologic outcomes [56]. A study of approximately 9,000 adults on ART in South Africa found lower rates of virologic suppression in younger adults (16-24 years) compared with those aged 25-49 [57]. These data suggest that younger age predicts failure to achieve viral suppression, and that this risk decreases with age. Younger age is associated with poor ART adherence [58-60]. Younger age also predicted low PrEP adherence in our cohort [61]. Behavioral and psychosocial correlates of nonadherence in young adults include anxiety and depression [48, 62], HIV-1 associated stigma and discrimination, lack of disclosure, feelings of invulnerability to the consequences of HIV-1 disease [44, 63], alcohol and recreational drug use [62], and low socioeconomic status [64]. These factors may

mediate the observed association between younger age, nonadherence and poor virologic outcomes.

Our finding that younger age predicts virologic rebound is consistent with prior studies that reported lower risk of viral rebound with older age [65, 66], and a significantly shorter time to viral rebound in younger adults (20-29 years) compared with older (≥ 30 years) [67]. In a prospective study of 1305 HIV-1 infected persons on ART in British Columbia, younger age was independently associated with viral rebound after initial viral suppression [68]. Predictors of nonadherence in sub-Saharan Africa include higher pill burden (twice-daily vs. once-daily regimens) [69], stavudine-containing regimens [70], geographic or transportation-related barriers [71] and GPS measured distance from home to clinic [72]. In multiple settings, younger age predicts virologic rebound and clinicians and ART programs should more effectively address behavioral, structural and psychosocial barriers to ART adherence and provide adherence support for younger HIV-infected persons initiating ART.

We previously reported that younger age predicted delayed ART initiation in this cohort [51]. In contrast, a South African study found no age difference between ART initiators and refusers [73]. Delayed ART initiation is common in sub-Saharan Africa [74-77], and results from health system, provider, and patient-level factors [76, 78-81]. Individuals who delay ART initiation may not be motivated to achieve the high-level of adherence needed to achieve and sustain viral suppression [82] or may have competing priorities, less stable lives and less familiarity or experience with daily treatment. Our finding that younger age predicts ART non-initiation, delays in achieving viral suppression and virologic rebound points to a common theme related to ART barriers. Patient-level barriers to ART initiation are often similar to adherence barriers and include denial of the need to start treatment, lack of motivation to stay on treatment, fear of side effects, lack of social support, stigma, fear of disclosure, and the perception that starting ART signifies AIDS and impending mortality [76, 81-87]. Lower mental health scores were the only psychosocial variable significantly associated with poor adherence in a randomized trial of ART for HIV-1 prevention (HPTN 052) [88]. These factors may influence adherence during the initial period after ART initiation as well as subsequent virologic outcomes. Durable viral load suppression is the ultimate goal of ART, both for individual health outcomes and for treatment as prevention. Addressing adherence

barriers is key to improving virologic outcomes in younger adults. Evidence-based interventions to improve adherence include once-daily regimens, fixed dose combinations, reminder devices, mobile health technology, and provision of one-on-one or couple-based adherence support with feedback about viral suppression [88, 89].

The strengths of our study include the prospective design, large sample size, the diversity of a multinational cohort specifically of HIV-1 infected persons with known HIV-1 uninfected partners, regular clinical and laboratory monitoring of HIV-1 infected participants, and robustness of results in sensitivity analyses. Limitations of the analysis include limited follow-up time after ART initiation, lack of in-depth psychometric surveys to understand the bases for adherence and non-adherence, and reasons for non-adherence in real time, since viral load testing was conducted after the end of the study. We evaluated participants who were alive and on treatment at the time viral suppression was assessed (on-treatment analysis), and may have overestimated the effectiveness of ART. However, nearly all HIV-1 infected partners were retained in study follow-up, and thus our results are unlikely to be influenced by selection bias due to attrition and death.

In conclusion, the majority of heterosexual East African HIV-1 infected persons with known HIV-1 uninfected partners achieved viral suppression. Younger age independently predicted delayed ART initiation, failure to achieve viral suppression and virologic rebound in this cohort. ART programs should give special consideration to motivating adherence and sustaining viral suppression in younger HIV-1 infected persons in serodiscordant partnerships. Future studies should develop, test, implement and rigorously evaluate evidence-based interventions to improve ART adherence in young adults, and assess their correlation with virologic outcomes.

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Author contributions

AM and JMB designed the study and wrote the first draft. AM performed the statistical analyses. All authors contributed to data collection, interpretation of the results and the writing of the manuscript, and all approved the final draft.

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Role of the funding source

The authors designed and executed the study, had full access to the raw data, performed all analyses, wrote the manuscript, and had final responsibility for the decision to submit for publication. The funder had no role in design, data collection, analysis, interpretation, or writing of the report.

Partners PrEP Study Team:

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Data management was provided by DF/Net Research, Inc. (Seattle, USA) and site laboratory oversight was provided by Contract Laboratory Services (CLS) of the Wits Health Consortium (University of the Witwatersrand, Johannesburg, South Africa).

Study medication was donated by Gilead Sciences.

Figure 1. Study Profile of HIV-1 infected persons enrolled in the Partners PrEP Study

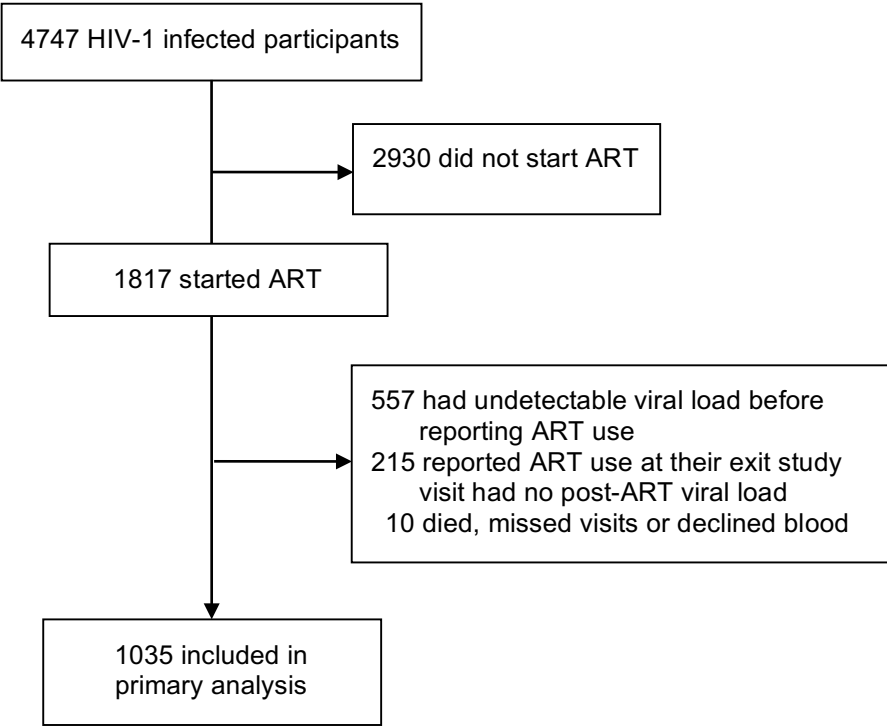


Figure 2. Time to first viral suppression following ART initiation

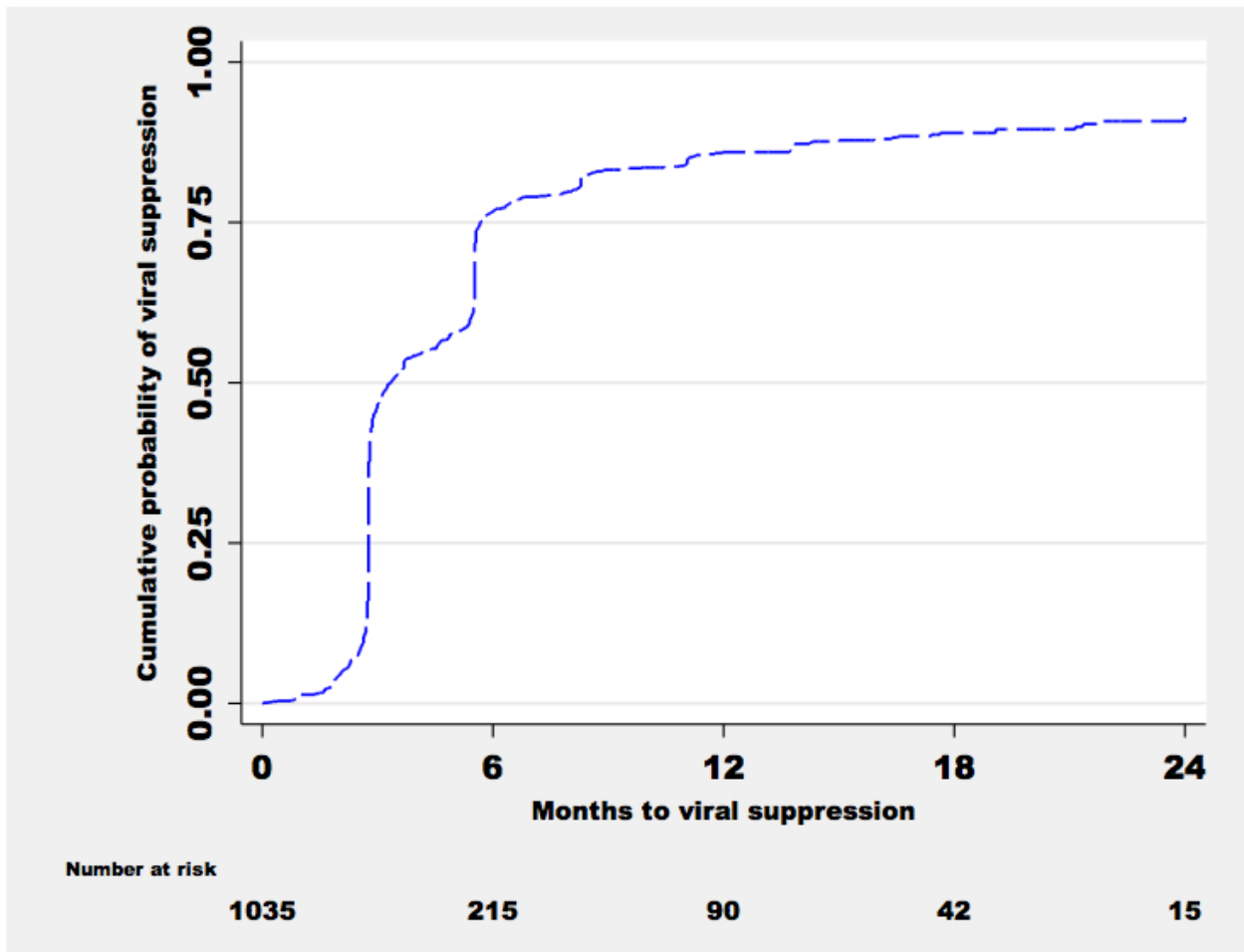


Figure 3. Cumulative probability of virologic rebound following initial viral suppression

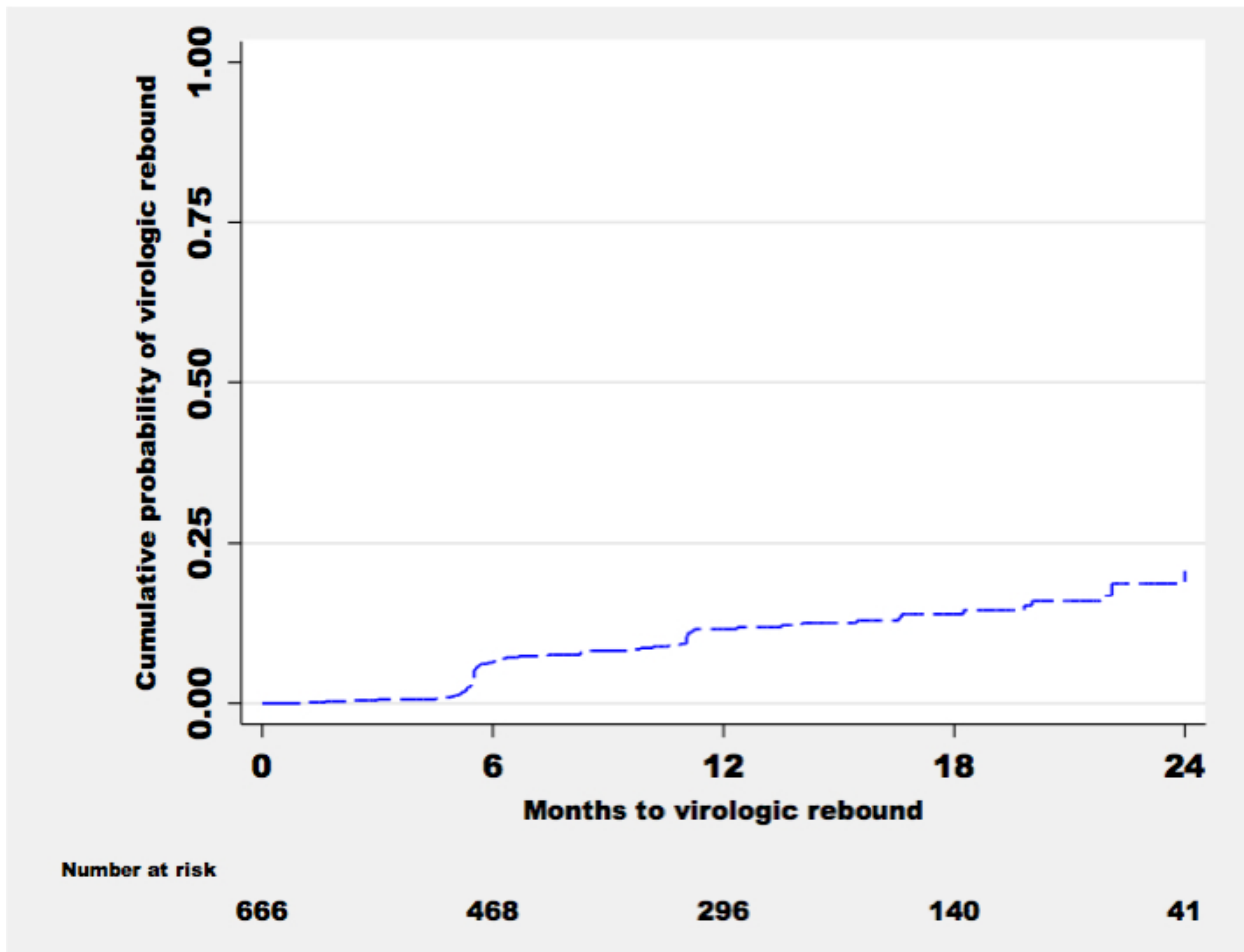


Table 1. Participant characteristics and factors associated with viral non-suppression

Covariate	N (%) or median (IQR)	Hazard Ratio (95% CI)	P-value	Adjusted Hazard Ratio (95% CI)	P-value
Age (per 5 years younger)	35 (28-41)	1.05 (1.01-1.09)	0.01	1.05 (1.04-1.10) ⁺	0.006
Gender					
Women	560 (54)	Referent			
Men	475 (46)	0.97 (0.85-1.10)	0.63
Partnership duration					
≥ 1 year	961 (93)	Referent		Referent	
< 1 year	74 (07)	1.19 (0.91-1.56)	0.19	1.19 (0.91-1.55)	0.21
Known HIV discordance					
≥1 year	430 (42)	Referent			
<1 year	605 (58)	1.06 (0.93-1.21)	0.41
Education (years)					
>7	396 (38)	Referent			
≤7	639 (62)	1.02 (0.89-1.17)	0.74
Visits at which unprotected sex acts were reported *					
None	1264 (88)	Referent			
Any	169 (12)	0.90 (0.73-1.10)	0.29
Visits when pregnant *					
No	1363 (96)	Referent			
Yes	53 (04)	1.18 (0.82-1.70)	0.37
Children in partnership					
Any	839 (81)	Referent			
None	196 (19)	0.97 (0.81-1.14)	0.70
Monthly income					
Any	727 (70)	Referent		Referent	
None	308 (30)	0.90 (0.77-1.04)	0.14	1.14 (0.97-1.33)	0.11
Alcohol consumption					
None	832 (80)	Referent			
Any	203 (20)	0.94 (0.80-1.10)	0.43
Pre-treatment CD4 count					
>250 cells/μL	500 (48)	Referent		Referent	
≤250 cells/μL	535 (52)	1.28 (1.12-1.46)	0.003	1.26 (1.10-1.45)	0.009
Pre-treatment HIV-1 RNA (per log ₁₀ copies/mL)	4.44 (3.90-4.89)	1.23 (1.12-1.34)	<0.001	1.21 (1.11-1.33)	<0.001

* Pregnancy and unprotected sex were analyzed as time varying covariates. These are visits at which unprotected sex was reported or the woman was pregnant and blood was collected for plasma viral load quantification.

+ The adjusted hazard ratio for younger age differed by <1% after stratifying by study site (aHR 1.052).

Table 2. Sensitivity Analyses: Predictors of failure to achieve viral suppression

Covariate	HIV-1 RNA >400 copies/mL		All who achieved viral suppression (>80 copies/mL)	
	Adjusted Hazard Ratio (95% CI)	P-value	Adjusted Hazard Ratio (95% CI)	P-value
Age (per 5 years younger)	1.04 (1.01-1.09)	0.01	1.08 (1.03-1.11)	<0.001
Partnership duration ≥ 1 year < 1 year	Referent 1.19 (0.92-1.53)	0.18	Referent 1.17 (0.92-1.49)	0.21
Monthly income Any None	Referent 1.15 (0.99-1.34)	0.06	Referent 1.12 (0.98-1.28)	0.11
Pre-treatment CD4 count >250 cells/μL ≤250 cells/μL	Referent 1.21 (1.06-1.37)	0.004	Referent 1.21 (1.07-1.38)	0.002
Pre-treatment HIV-1 RNA (per log ₁₀ copies/mL)	1.12 (1.03-1.23)	0.008	2.19 (2.04-2.34)	<0.001

Chapter 3. Seminal HIV-1 RNA detection in heterosexual African men initiating antiretroviral therapy

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**Seminal HIV-1 RNA Detection in Heterosexual African Men Initiating
Antiretroviral Therapy**

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ABSTRACT

Background: Eliminating HIV-1 transmission using antiretroviral therapy (ART) requires suppressing HIV-1 RNA in blood and genital secretions. However, intermittent HIV-1 semen shedding has been described despite effective ART and suppressed blood HIV-1 RNA concentrations.

Methods: We used data from a prospective study of heterosexual HIV-1 infected men initiating ART to assess the frequency, magnitude and correlates of seminal HIV-1 RNA shedding. HIV-1 RNA was quantified in semen and blood using the Abbott m2000 Real-Time HIV-1 assay (Abbott Diagnostics) with a lower limit of quantification of 40 copies/mL. The primary outcome was detectable semen HIV-1 RNA concentrations.

Results: 231 men who initiated ART during follow-up provided 274 semen samples. Overall, seminal HIV-1 RNA was detected in 18% (50/274) of semen samples: 24% (37/155), 10% (5/49) and 11% (8/70) of samples collected after 0-3, 4-6 and >6 months of ART, respectively. Paired blood and semen HIV-1 RNA concentrations were strongly correlated (Spearman's $\rho=0.63$; $p<0.001$). Seminal HIV-1 RNA was detected in 8% (16/195) of samples collected when blood HIV-1 RNA concentrations were suppressed, at seminal HIV-1 RNA copy numbers between 80 and 2796 copies/mL and most (13/16, 82%) had detectable semen HIV-1 RNA <1000 copies/mL.

Conclusions: HIV-1 RNA semen shedding was infrequent and of low quantity in African HIV-1 infected men who achieved HIV-1 RNA suppression in blood.

Background

Semen is the principal vector for transmitting human immunodeficiency virus type 1 (HIV-1) from men to women [7]. Combination antiretroviral therapy (ART) suppresses HIV-1 RNA concentrations in both blood and semen below the lower limit of detection for commercially available assays, which substantially decreases the risk of sexual HIV-1 transmission to uninfected partners [90]. First-phase HIV-1 RNA decay is characterized by rapid clearance of free virus and short-lived productively infected cells during the first 7-10 days of ART. Second-phase decay, with an average half-life of 14 days, features removal of long-lived memory T-cells, dendritic cells and macrophages [91]. First-phase decay kinetics may be slower in semen than in blood, but second-phase decay rates are thought to be similar [92].

Three HIV-1 shedding patterns have been described in semen: none, continuous and intermittent [93]. In men who shed HIV-1 in semen, the predominant shedding pattern is intermittent [93]. Several prospective studies of men with suppressed blood HIV-1 RNA levels have described intermittent HIV-1 semen shedding, even in the absence of sexually transmitted infections (STIs) [94]. In men with low seminal viral loads, a probabilistic empiric model estimated a male-to-female HIV-1 transmission risk of 3/10,000 sexual episodes [95].

HIV-1 infected men initiating ART require counseling about risk of male-to-female HIV-1 transmission, including information about timing and likelihood of achieving HIV-1 RNA suppression in semen. In a large prospective study of heterosexual HIV-1 serodiscordant African couples, we assessed the frequency, magnitude and correlates of seminal HIV-1 RNA shedding in men initiating ART.

Methods

Study population

Study participants were heterosexual HIV-1 infected African men who initiated ART between July 2008 and December 2012 in Kenya and Uganda. The study cohort was derived from HIV-1 serodiscordant couples enrolled in the Partners PrEP Study, a randomized clinical trial of daily oral antiretroviral pre-exposure prophylaxis [52]. At enrollment, HIV-1 infected partners did not meet eligibility criteria for ART

initiation according to national treatment guidelines. They were followed quarterly, and received regular clinical monitoring, 6-monthly CD4 counts and counseling about ART benefits. Those who became eligible for ART were referred to collaborating HIV-1 clinics for treatment. ART use was assessed every three months.

All men were provided with HIV-1 prevention services including risk-reduction counseling, free condoms, and screening and treatment of sexually transmitted infections according to World Health Organization guidelines. Ethics review committees at collaborating institutions at each of the study sites and the University of Washington Human Subjects Review Committee approved the research protocol. Study participants provided independent written informed consent in English or their local language.

Clinical and laboratory procedures

Blood and semen collection were scheduled at regular intervals following study enrollment, and not according to the time of ART initiation. HIV-1 infected men provided blood for HIV-1 RNA quantification at enrollment, every six months thereafter, and at the last study visit. CD4 cell counts were quantified every six months using flow cytometry (BD Biosciences). Semen collection was scheduled at the six and twelve month study visits, but if missed, samples could be collected at any subsequent visit when convenient. Men who agreed to give a semen sample were provided with a sterile plastic container and spermicide-free condoms. Semen was obtained by masturbation at the study clinic, or during coitus at home after 48-72 hours of sexual abstinence. Semen specimens were transported to the study clinic within 5 hours of collection. Following liquefaction and centrifugation for 10 minutes at 600-800g, seminal plasma was recovered, stored at -70°C and shipped on dry ice (along with frozen blood plasma) to the University of Washington Retrovirology Laboratory (UW RVL) for HIV-1 RNA quantification. The UW RVL is CLIA compliant and College of American Pathologists certified. Blood and semen HIV-1 RNA concentrations were quantified using the Abbott m2000 Real-Time HIV-1 assay (Abbott Diagnostics); the lower limit of detection was 40 copies/mL. For the present study, all semen samples collected following initiation of ART were tested.

At enrollment, every 12 months thereafter and when clinically indicated, urine samples were tested for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using APTIMA Combo 2 (Gen-Probe) or COBAS AmpliCor (Roche Diagnostics) assays, and for *Trichomonas vaginalis* infection using APTIMA TV TMA (Gen-Probe) or In Pouch TV (Biomed Diagnostics). Syphilis serology was performed using rapid plasma reagin tests and positive results confirmed using *Treponema pallidum* particle agglutination tests. All study site laboratories participated in external quality assurance programs for the respective testing.

Data Analysis

All HIV-1 infected men who initiated ART and provided a semen sample were included in the analysis. The primary outcome was detectable semen HIV-1 RNA levels. HIV-1 RNA levels were assessed in paired blood and seminal plasma samples, and if paired samples were not available, in blood samples drawn within 3 months of the semen sample. Presence of HIV-1 RNA in semen was quantified in three time periods: 0-3, 4-6 and >6 months after ART initiation. The correlation between blood and seminal HIV-1 RNA quantity was assessed using Spearman's rank correlation coefficient. Factors potentially associated with seminal HIV-1 shedding were evaluated using generalized estimating equations with a logit link and independent correlation structure to account for multiple observations per person. Potential confounding factors known to be associated with ART adherence and seminal HIV-1 shedding were selected *a priori* and included age, sexually transmitted infections, pre-ART CD4 cell count, blood HIV-1 RNA concentrations, and time on ART. Factors with p-values ≤ 0.20 in bivariate analyses were included in the multivariate model. All analyses were performed using Statistical Analysis Software (SAS), version 9.4.

Results

We followed 1778 couples with HIV-1 infected male partners for 4554 person-years. Of the 755 HIV-1 infected men who initiated ART, 491 (65%) provided semen before ART and 31 (4%) did not give semen. The remaining 233 (31%) provided 280 semen samples after ART initiation. Six samples had failed runs and did not contribute to the analysis. Of the remaining 274 semen samples, 221 (81%) had a matching blood sample. Following ART initiation, 231 men were followed for a median of 1.6 years (interquartile

range [IQR] 1.2-2.1), and the median time from ART initiation to semen HIV-1 RNA quantification was 2.83 months (IQR, 0.16-6.45). Most (98%) were on ART regimens containing a non-nucleoside reverse transcriptase inhibitor.

Characteristics of HIV-1 infected men are shown in Table 3. The median age at enrollment was 40 years (IQR, 34-47), nearly all (99%) were married; the median CD4 cell count and HIV-1 RNA concentration were 336 cells/ μ L (IQR, 293-403) and 4.54 \log_{10} copies/mL (IQR, 3.81-5.03), respectively. The prevalence of sexually transmitted infections was <1%. The 233 men who had a semen sample collected after ART initiation were similar to the 522 men who did not provide a semen sample, except that they were less likely to have a monthly income (80 vs. 87%), had lower pre-treatment CD4 counts (244 vs. 272 cells/ μ L) and started ART earlier during study follow up (19.4 vs. 8.3 months).

HIV-1 semen shedding

Overall, HIV-1 RNA was detected in 18% (50/274) of semen samples. Seminal HIV-1 RNA detection ranged from 24% (37/155) for samples collected 0-3 months after ART initiation to 10% (5/49) of samples collected 4-6 months after ART initiation and 11% (8/70) of samples >6 months after ART initiation. When detected, median HIV-1 RNA levels were 2.96 \log_{10} copies/mL (IQR, 2.51-3.50).

Semen HIV-1 RNA was detected in 8% (16/195) of samples obtained when blood HIV-1 RNA concentrations were suppressed, specifically 11% (10/90), 5% (2/41) and 6% (4/64) of semen samples collected after 0-3, 4-6 and >6 months of ART, respectively. When blood HIV-1 was suppressed and semen HIV-1 RNA was detected, the median concentration of semen HIV-1 RNA was 2.52 \log_{10} copies/mL (IQR, 2.23-2.98). Most (82%, 13/16) had semen HIV-1 RNA quantity <1000 copies/mL. No STIs were detected at the time HIV-1 was detected in semen.

Factors associated with seminal HIV-1 RNA detection

Paired blood and semen HIV-1 RNA concentrations were strongly correlated (Spearman's rank correlation coefficient, $\rho=0.63$; $p<0.001$). In bivariate analyses, semen HIV-1 RNA shedding was

significantly associated with the concentration of HIV-1 RNA in blood ($p < 0.001$), pre-treatment CD4 count ($p = 0.04$), any monthly income ($p = 0.009$), and duration of ART ($p = 0.05$) (Table 4). After adjusting for potential confounding factors, the HIV-1 RNA concentration in blood significantly predicted HIV-1 RNA semen shedding (adjusted odds ratio [AOR] 2.72 per \log_{10} ; $p < 0.001$).

Discussion

This prospective analysis of sexually active HIV-1 infected men initiating ART is the largest study to determine the relationship between suppression of HIV-1 RNA in blood plasma and seminal HIV-1 RNA shedding in sub-Saharan Africa. HIV-1 semen shedding was infrequent and of low quantity when blood HIV-1 concentrations were suppressed. Most men who shed HIV-1 in their semen had HIV-1 RNA levels < 1000 copies/mL of seminal plasma and HIV-1 RNA levels in blood predicted HIV-1 semen shedding.

The frequency and magnitude of intermittent HIV-1 semen shedding we observed was similar to prospective studies of men on suppressive ART receiving medically-assisted reproduction services to initiate a pregnancy with HIV-1 uninfected women partners [96-98]. Among men with an undetectable HIV-1 level in blood, HIV-1 RNA was detected in the semen of 3.7 to 19.3% of European HIV-1 infected men in serodiscordant partnerships [96-100]. In our study, 8% of semen samples had detectable HIV-1 RNA when blood HIV-1 levels were suppressed. Of these 16 samples, 13 (82%) had semen RNA levels < 1000 copies/mL. This concentration of HIV-1 in semen is associated with very low risk of male-to-female HIV-1 transmission [7].

A rare male-to-male HIV-1 transmission event involving an HIV-1 infected partner with undetectable blood HIV-1 RNA levels has been reported in the literature [31]. We previously reported that there were no male-to-female HIV-1 transmission events in serodiscordant couples in which HIV-1 infected men started ART, despite self-reported unprotected sex with HIV-1 uninfected women partners and high pregnancy incidence [101]. These data support the HIV-1 prevention effectiveness of suppressive ART, regardless of low level viraemia infrequently detected in semen.

Our finding that HIV-1 was shed in semen in the absence of STIs is in agreement with prior studies of HIV-1 infected MSM on ART in which 6-8% had detectable HIV-1 RNA in semen despite suppressed HIV-1 levels in blood and absence of detectable STIs [94]. In those studies, cytomegalovirus co-infection and the size of the latent blood HIV-1 reservoir predicted seminal HIV-1 RNA shedding. Intermittent shedding of HIV-1 in semen may arise from stimulation of genital mucosa by concurrent STIs, genital inflammation, T-cell immune activation, reflect HIV-1 compartmentalization, or incomplete ART penetration of the male genital tract [94]. Recent work indicates that intermittent shedding of HIV-1 in semen can occur within a one-hour interval [100]. This variability may be similar to isolated episodes of low-level viremia, in which detectable blood HIV-1 RNA levels (typically HIV-1 RNA <400 copies/mL) occur after viral suppression and are followed by return to virological suppression.

The strength of our study is that we conducted the largest analysis of HIV-1 semen shedding in heterosexual African men on ART. A limitation of our study was that we only quantified cell-free HIV-1 total nucleic acid concentrations in seminal plasma. Measuring both cell-associated HIV-1 RNA and DNA may provide a more accurate measure of total HIV-1 levels in semen. However, quantifying HIV-1 total nucleic acid and cell-free HIV-1 DNA in seminal plasma is not routinely performed as a marker of HIV-1 replication in semen. STI prevalence in our cohort was low and our results may not be generalizable to high-risk populations. Nevertheless, most studies of seminal HIV shedding on suppressive ART report prevalence rates of 6-8%.

In conclusion, we observed a low prevalence and quantity of HIV-1 semen shedding in heterosexual HIV-1 infected African men on suppressive ART with undetectable HIV-1 levels in blood. This confirms the importance of ART for suppressing the frequency and level of HIV-1 nucleic acid shedding in seminal plasma.

Competing interests

The authors report no conflicts of interest.

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Role of the funding source: The authors designed and executed the study, had full access to the raw data, performed all analyses, wrote the manuscript and had final responsibility for the decision to submit for publication. The funder had no role in design, data collection, analysis, interpretation or writing of the report.

Author contributions

AM and JMB designed the study and wrote the first draft. RWC oversaw all HIV-1 RNA testing. AM performed the statistical analyses. All authors contributed to data collection, interpretation of the results and the writing of the manuscript and all approved the final draft.

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Study medication was donated by Gilead Sciences.

Table 3: Characteristics of 231 HIV-1 infected men

Characteristic	Median (IQR) or N (%)
Age (years)	40 (34-47)
Married to study partner	230 (99)
Education (>7 years)	134 (58)
Monthly income (any)	185 (80)
Unprotected sex (any)*	20 (9)
CD4 count (cells/ μ L)*	244 (212-313)
Blood HIV-1 RNA level (log ₁₀ copies/mL)*	4.54 (3.81-5.03)
Sexually transmitted pathogen*	
<i>Trichomonas vaginalis</i>	0 (0)
<i>Neisseria gonorrhoeae</i>	2 (<1)
<i>Chlamydia trachomatis</i>	0 (0)
<i>Treponema pallidum</i>	2 (<1)
Duration of ART (months)	19.42 (14.0-24.7)

* Time-varying covariates were assessed at the study visit prior to ART initiation

Table 4. Associations with seminal HIV-1 RNA detection

Variable	Odds ratio (95% CI)	P-value	Adjusted odds ratio (95% CI)	P-value
Age (per year)	1.03 (0.99-1.07)	0.13	1.05 (0.99-1.10)	0.08
Monthly income (any)	2.57 (1.27-5.20)	0.009	1.96 (0.74-5.17)	0.17
Duration of ART (per month)	0.91 (0.83-1.00)	0.05	0.95 (0.86-1.04)	0.28
Pre-ART CD4 (per 100 cells/ μ L)	1.78 (1.02-3.12)	0.04	1.92 (0.97-3.82)	0.06
Blood HIV-1 RNA level (per \log_{10} copies/mL)	2.56 (1.79-3.66)	<0.001	2.72 (1.79-4.14)	<0.001
<i>Neisseria gonorrhoeae</i>	1.35 (0.66-2.74)	0.41
<i>Trichomonas vaginalis</i>	1.38 (0.67-2.84)	0.39

Chapter 4. HIV transmission risk persists during the first 6 months of antiretroviral therapy

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Citation:

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HIV-1 Transmission Risk Persists During the First 6 Months of Antiretroviral Therapy

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ABSTRACT

Objective: Combination antiretroviral therapy (ART) decreases the risk of sexual HIV-1 transmission by suppressing blood and genital HIV-1 RNA concentrations. We sought to determine HIV-1 transmission risk prior to achieving complete viral suppression.

Design: Prospective cohort study.

Methods: Using data from the Partners PrEP Study, a prospective study of 4747 heterosexual HIV-1 serodiscordant couples in Kenya and Uganda, we examined multiple markers of HIV-1 transmission risk during the first months after ART initiation: time to viral suppression in blood, persistence of HIV-1 RNA in genital specimens, sexual risk behavior, pregnancy incidence, and HIV-1 transmission using survival analysis and GEE logistic regression.

Results: The cumulative probabilities of achieving blood viral suppression (<80 copies/mL) 3, 6 and 9-months after ART initiation were 65.3%, 84.8% and 89.1%, respectively. Endocervical and seminal HIV-1 RNA were detectable in 12% and 21% of samples obtained within 6-months of ART. Pregnancy incidence was 8.8 per 100 person-years during the first 6-months of ART, and sex unprotected by condoms was reported at 10.5% of visits. Among initially uninfected partners, HIV-1 incidence before ART was 2.08 per 100 person-years (55 infections; 2644 person-years), 1.79 for 0-6 months after ART initiation (3 infections; 168 person-years), and 0.00 with >6 months of ART (0 infections; 167 person-years).

Conclusions: Residual HIV-1 transmission risk persists during the first 6-months of ART, with incomplete viral suppression in blood and genital compartments. For HIV-1 serodiscordant couples in which the infected partner starts ART, other prevention options are needed, such as pre-exposure prophylaxis, until viral suppression is achieved.

Introduction

Blood and genital HIV-1 RNA concentrations are the prime determinant of HIV-1 transmission risk [6, 7]. Combination antiretroviral therapy (ART) prevents sexual transmission of HIV-1 by suppressing HIV-1 RNA in blood and genital secretions [4]. After ART initiation, the blood concentration of HIV-1 RNA decreases in multiple overlapping phases [91]. During the first two phases of decay, blood viremia declines rapidly due to clearance of free virus, macrophages and productively infected and partially activated CD4+ T-cells, but HIV-1 RNA is still detectable, suggesting residual risk of HIV-1 transmission. In the third and fourth phases of decay, blood HIV-1 RNA concentrations decline below the limit of quantification of standard assays [102, 103]; typically, viral suppression occurs within six months after initiation of combination ART [5].

Understanding the potential for HIV-1 infectiousness during the period between ART initiation and complete viral suppression is a priority for patients, providers, and public health policymakers. As ART for HIV-1 prevention is scaled up, data are needed to assess ART effectiveness during this risk period when sexual partners remain at risk of HIV-1 acquisition. Within a prospective study among HIV-1 serodiscordant couples, we evaluated measures of HIV-1 transmission risk after ART initiation, focusing on residual risk during the first 6 months of ART, when the majority of patients typically achieve viral suppression.

Methods

Study population

This analysis utilized data from the Partners PrEP Study, a randomized clinical trial of daily oral antiretroviral pre-exposure prophylaxis (PrEP) to decrease HIV-1 acquisition in heterosexual serodiscordant couples [53]. As previously reported, 4747 HIV-1 serodiscordant couples from Kenya and Uganda were followed between 2008 and 2012 [52]. At study entry, couples were sexually active and planning to remain as a couple for the duration of the study. HIV-1 infected partners were not eligible for ART according to national guidelines at the time of enrollment. During follow-up, they received regular clinical and immunological monitoring and referrals for ART if they became eligible for treatment, initially

at CD4 <200 cells/ μ L (Kenya) and <250 cells/ μ L (Uganda), which was revised to \leq 350 cells/ μ L in both countries while the study was ongoing. ART use by HIV-1 infected partners and sexual behavior as reported by both members of the couple were assessed every three months.

All participants received a package of HIV-1 prevention services including individual and couple risk-reduction counseling, free condoms, and screening and treatment of sexually transmitted infections. At all study sites, HIV-1 uninfected women were provided with contraception counseling and free contraceptives. Institutional review board approval was obtained from each collaborating institution and the University of Washington. All participants provided written informed consent in English or their local language.

Specimen collection and processing

At enrollment, every six months and at study exit, blood was collected from HIV-1 infected partners for HIV-1 RNA quantification. Thus, blood was collected on a schedule relative to enrollment, not to ART initiation. Similarly, cervical specimens were collected at enrollment, annually and study exit. Swabs were collected by placing Dacron swabs into the endocervical canal and gently rotating twice, then were snipped distal from the base of the swab, inserted in aliquot vials, and frozen at -70°C within 5 hours of collection. Semen collection was scheduled at the six and twelve month visits, but samples could be obtained at any visit thereafter, depending on the preference of the male subject. At the prior visit, men were provided with a spermicide-free condom and sterile wide-mouth plastic container and instructed to abstain from ejaculation for 48-72 hours. Semen was collected during coitus on the day of the clinic visit, or through masturbation at the clinic or participant's home, and brought into clinic within 5 hours of collection. Semen was centrifuged at 600-800g for 10 minutes within 4 hours of arrival in the laboratory. Seminal blood was separated from the cell pellet, aliquoted into cryovials and stored at -70°C [104].

HIV-1 serological testing for HIV-1 uninfected partners was performed monthly using dual rapid HIV-1 antibody tests, and positive results confirmed by HIV-1 EIA, Western blot and RNA polymerase chain reaction (PCR) [55]. Phylogenetic linkage between HIV-1 seroconverters and their study partners was ascertained using HIV-1 *pol* gene consensus sequencing. We used the Abbott m2000 Real-Time HIV-1

assay (Abbott Diagnostics) to quantify HIV-1 RNA in blood and genital samples. The lower limit of detection was 40 copies/mL in blood and semen plasma and 248 copies/swab in endocervical secretions. Urine pregnancy testing was conducted monthly for HIV-1 uninfected women and when clinically indicated for HIV-1 infected women.

Statistical analysis

To assess ongoing HIV-1 transmission risk after ART initiation, we evaluated several measures: time to first viral suppression in blood, persistence of HIV-1 RNA in genital secretions, self-reported sexual behavior, incidence of pregnancy, and phylogenetically linked HIV-1 transmission within the couple. For analyses of viral suppression, sexual risk behavior and pregnancy incidence, we evaluated couples in which the HIV-1 infected partner initiated ART (N=1817). Analysis of HIV-1 transmission was restricted to couples in the trial's placebo arm, including those who did and did not initiate ART (N=1573). Blood viral suppression was defined as HIV-1 RNA concentrations <40 copies/mL. Follow-up time was computed beginning on the date of the study visit at which ART use was first reported (thus, after ART had started). Kaplan-Meier methods were used to estimate the cumulative proportion achieving viral suppression. Persistence of HIV-1 RNA in genital samples was described as the proportion with detectable HIV-1 RNA.

Sexual risk behavior, pregnancy incidence and HIV-1 transmission risk were quantified in three time periods: before ART initiation, during the first six months of ART, and after six months of ART. The frequency of self-reported condomless sex was calculated as the proportion of visits at which condomless sex was reported. Differences in the proportion of condomless sex between ART time periods were estimated using logistic regression with generalized estimating equations. Pregnancy incidence was computed by dividing the number of new pregnancies by follow up time at risk for pregnancy. Pregnancy and HIV-1 incidence rates and 95% confidence intervals were estimated using exact methods assuming a Poisson distribution. All analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC) and Stata 12.1 (StataCorp, College Station, TX).

Results

Participant characteristics

Of the 4747 HIV-1 infected persons followed in the Partners PrEP Study, 2184 were eligible for ART of which 1817 (83%) initiated ART (1062 women and 755 men). Most (98%) were married and the median ages of HIV-1 infected men and women were 40 years (interquartile range [IQR], 35-45) and 30 years (IQR, 25-36), respectively. At the study visit prior to reporting ART use, the median CD4 count and blood HIV-1 RNA concentration were 288 cells/ μ L (IQR, 226-390) and 4.03 log₁₀ copies/mL (IQR, 3.36-4.61) for HIV-1 infected women, and 262 cells/ μ L (IQR, 216-339) and 4.39 log₁₀ copies/mL (IQR, 3.75-4.90) for HIV-1 infected men ($p < 0.001$ for each variable comparing women and men).

Blood HIV-1 RNA suppression

Of the 1817 ART initiators, 215 who reported ART use at the exit study visit and 10 who died or missed study visits after starting ART did not contribute to the analysis. The remaining 1592 were followed for 474 person-years to assess time to first viral suppression. The median time from date of the study visit at which ART use was first reported until first blood HIV-1 RNA quantification was 3.1 months (IQR, 2.8-5.5). The cumulative probabilities of achieving blood viral suppression 3, 6, 9 and 12 months after the reported date of ART initiation were 65.3%, 84.8%, 89.1% and 90.9%, respectively (Figure 1).

Genital HIV-1 RNA levels

Of the 1062 women ART initiators, endocervical HIV-1 RNA concentrations were available for 929 (87%). Of these, 492 women (53%) contributed one sample, 267 women (29%) contributed two samples, 149 women (16%) contributed three samples and 21 (2%) women contributed four samples. The median time from the reported date of ART initiation to the first endocervical HIV-1 RNA quantification was 3.7 months (IQR, 0.4-8.3). During the first six months of ART, endocervical HIV-1 RNA was detected in 12% (75/625) of swabs (Table 5) and the median quantity was 3.11 log₁₀ copies/swab (IQR, 2.59-3.62). Among 454 swabs collected when blood HIV-1 RNA concentrations were undetectable (<40 copies/mL), endocervical HIV-1 RNA was detected in 8% (36 swabs from 36 women), and the median quantity was 3.14 log₁₀ copies/swab (IQR, 2.88-3.53). When blood HIV-1 RNA concentrations were detectable, endocervical HIV-1 RNA was detected in 23% (39/171) of swabs.

Of the 755 male ART initiators, 231 (31%) had semen samples available for HIV-1 RNA quantification after initiating ART; 189 men (82%) contributed one sample and 41 (18%) contributed 2 samples. The median time from ART start to seminal HIV-1 RNA quantification was 2.8 months (IQR, 0.0-4.6). During the first six months of ART, 21% (42/204) had detectable HIV-1 RNA concentrations and the median quantity was 2.90 log₁₀ copies/mL (IQR, 2.51-3.50). Among 132 semen samples collected after blood HIV-1 RNA concentrations were suppressed, 9% (12 samples from 10 men) had detectable seminal HIV-1 RNA and the median quantity detected was 2.60 log₁₀ copies/mL. When blood HIV-1 RNA concentrations were detectable, seminal HIV-1 RNA was detected in 42% (30/72) of swabs.

Sexual risk behavior

The frequency of self-reported condomless sex with HIV-1 infected partners was 12.8% (15207/118499 visits) before ART initiation, 10.5% (882/8386 visits) during the first six months of ART and 9.1% (1038/11446 visits) after six months of ART (p=0.008 for 0-6 months of ART versus no ART). The pregnancy incidence rate among HIV-1 infected and uninfected women partners was 13.2 per 100 person-years (1215 pregnancies; 9191 person-years) prior to ART initiation, 8.8 per 100 person-years (79 pregnancies; 895 person-years) during the first six months of ART, and 8.0 per 100 person-years (95 pregnancies; 1183 person-years) after six months of ART (p=0.004 for 0-6 months of ART versus no ART).

HIV-1 incidence

We followed 1573 HIV-1 serodiscordant couples enrolled in the placebo arm of the Partners PrEP Study for 2979 person-years. The HIV-1 incidence rate among couples not on ART was 2.08 per 100 person-years (55 infections; 2644 person years), and 1.79 per 100 person-years (3 infections; 168 person years) during the first six months of ART use. There were no HIV-1 transmissions during 167 person-years of follow up among couples exposed to ART for more than 6 months of ART (incidence rate 0.00; 95% CI: 0.00-2.20). All three ART-exposed HIV-1 events were phylogenetically-linked female-to-male transmissions and occurred prior to complete viral suppression in blood and genital secretions. For one

couple, the HIV-1 infected woman first reported ART use at the same study visit that her male partner tested seropositive for HIV-1. In the other two couples, HIV-1 seroconversion occurred within six months after first report of ART use (Table 6).

Discussion

HIV-1 transmission risk is markedly reduced once effective ART has resulted in complete virologic suppression in blood and genital secretions. However, this prospective follow up of 1592 HIV-1 serodiscordant couples after ART initiation by the HIV-1 infected partner demonstrates residual risk of HIV-1 transmission during the first six months of ART, as measured by HIV-1 in blood and genital secretions, behavioral risk, and direct measures of HIV-1 transmission with three phylogenetically-linked transmissions occurring soon after the HIV-1 infected partner reported ART initiation. Thus, the first 6 months after ART initiation may be a period of transition and persistent risk, with declining markers of transmission but not yet minimized risk.

Rigorous clinical studies have demonstrated that ART significantly reduces HIV-1 transmission risk in serodiscordant couples. A randomized trial and 5 observational studies followed 1672 serodiscordant couples for 5336 person-years [12, 14, 15, 17, 105, 106]. Six phylogenetically-linked HIV-1 transmission events were observed, of which at least 5 occurred within six months of ART initiation and the other occurred in the first year of ART [107]. In our study, all three men who acquired HIV-1 did so within six months of the first report of ART use, when their female HIV-1 infected partners still had detectable blood and genital HIV-1 RNA concentrations. One of these HIV-1 transmission events was observed at the same visit ART use was first reported, and soon after ART was initiated. The HIV-1 infected partner's blood and endocervical HIV-1 RNA concentrations at this visit were 56168 copies/mL and 3166 copies/swab, respectively. Twenty eight days later, her blood HIV-1 RNA concentration was 404 copies/mL, consistent with first and second phase decay kinetics when HIV-1 is still detectable, and infectious.

HIV-1 incidence during the first six months of ART use was similar to that among uninfected partners of

HIV-1 infected persons not yet on ART. The effectiveness of ART for HIV-1 prevention in our study is consistent with the PARTNER study, a European multi-center observational study of HIV-1 transmission from infected partners on suppressive ART, in which no transmissions occurred during 894 couple-years of follow up [108]. The precision of the upper bound of HIV-1 transmission risk on ART would be increased with additional HIV-1 transmission events and person-years of follow up [109].

The cumulative probabilities of achieving blood viral suppression three, six and nine months after starting ART were 65%, 85%, and 89%, respectively, suggesting residual transmission risk soon after ART initiation. Importantly, the majority of HIV-1 infected partners achieved complete viral suppression by six months after ART initiation. The fraction achieving viral suppression after 12 months in our study is similar to other cohorts from sub-Saharan Africa in which ~85% were fully suppressed after one year on ART [14, 106].

We observed ongoing high-risk sexual behavior during the first six months of ART as shown by self-reported sex unprotected by condoms and pregnancy incidence. The proportion reporting condomless sex in our study is comparable with that reported from a West African cohort in which 10-13% of HIV-1 infected persons on ART reported sex unprotected by condoms with serodiscordant partners [110]. The observed incidence of pregnancy in mutually-disclosed serodiscordant couples with access to comprehensive HIV-1 prevention services is comparable to annual pregnancy rates reported in other African cohorts [111, 112]. HIV-1 serodiscordant couples may practice condomless sex because of desire for children, condom fatigue or perception of low risk of HIV-1 transmission in dyadic heterosexual partnerships [113, 114].

Our results suggest that strategies to reduce HIV-1 risk prior to complete viral suppression are needed. Combining ART and PrEP for HIV-1 prevention results in near elimination of HIV-1 transmission in African serodiscordant couples [115]. As ART for HIV-1 prevention is scaled up worldwide, and couples are made aware of the treatment and prevention benefits of ART, providers should counsel serodiscordant couples about residual risk of HIV-1 transmission during the first six months of ART, and encourage use of

additional HIV-1 prevention services, including PrEP.

The strengths of our study include the large prospective multi-national cohort, the serodiscordant couple design permitting assessment of sexual behavior, HIV-1 infectiousness and phylogenetic linkage of HIV-1 transmission events to avoid misclassification of ART effectiveness. Our study has limitations. We relied on self-report of ART use at quarterly scheduled visits, and cannot precisely estimate the interval between ART start and HIV-1 transmission to susceptible partners. Most of the person-time at risk in our study was accrued before ART initiation, limiting the precision of the upper bound of our point estimate for HIV-1 transmission risk on ART. Viral load testing was done after the study ended, and participants were not provided with results in real-time, as routine viral load testing was not standard of care in the study settings.

In conclusion, among African HIV-1 serodiscordant couples, we observed residual risk of HIV-1 transmission, measured through virologic and behavioral outcomes, during the first six months of ART. During the transition to ART, other prevention options such as PrEP are needed for HIV-1 serodiscordant couples in which the infected partner delays, declines or is starting treatment. Ongoing studies are designed to provide further evidence of ART effectiveness for HIV-1 prevention.

Author contributions

AM and JMB designed the study and wrote the first draft. AM performed the statistical analyses. All authors contributed to data collection, interpretation of the results and the writing of the manuscript, and all approved the final draft.

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Figure 4. Cumulative probability of first plasma viral suppression from date of visit at which ART use was first reported

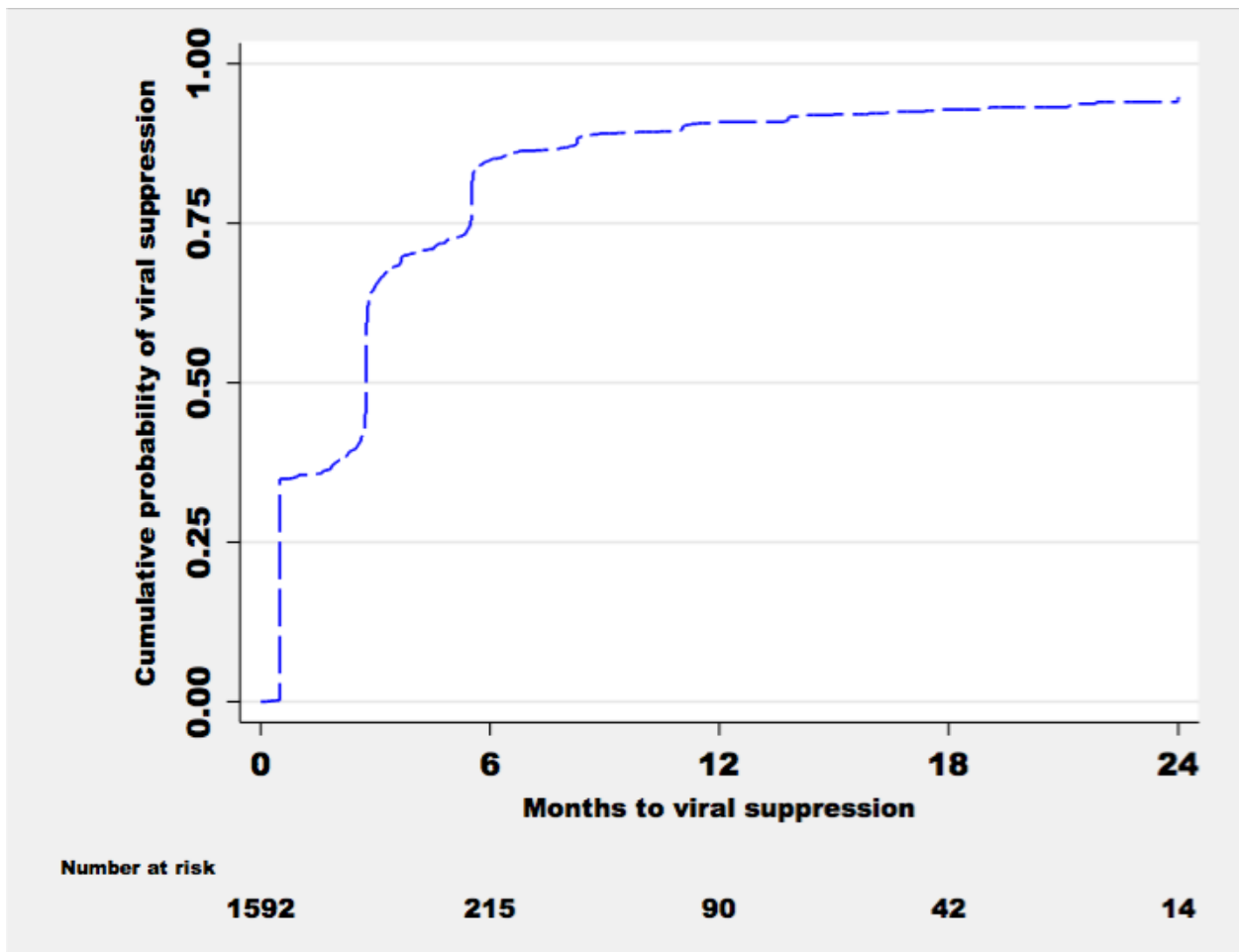


Table 5. Detection of HIV-1 RNA in blood and genital secretions during the first six months of ART

Blood HIV-1 RNA			
Genital HIV-1 RNA	Detected	Not detected	Total
Endocervical swabs			
Detected	39	36	75
Not detected	132	418	549
Total	171	454	625
Semen			
Detected	30	12	42
Not detected	42	120	162
Total	72	132	204

Table 6: Characteristics of women who transmitted HIV-1 to male partners while on ART

Variable	Couple 1	Couple 2	Couple 3
Couple demographics (age, gender)	40M, 50F	53M, 31F	32M, 29F
Enrollment plasma HIV-1 RNA (copies/mL)	332,514	20,188	694
Pre-ART plasma HIV-1 RNA (copies/mL)	1,434,082	56,168	824
Time from enrollment to ART initiation (months)	14	22	25
ART regimen	AZT/3TC/NVP	TDF/3TC/NVP	d4T/3TC/NVP
Time from reported date of ART initiation to HIV-1 seroconversion (days)	56	0	149
Time from reported date of ART initiation to HIV-1 RNA quantification (days)	84	28	86
Post ART plasma HIV-1 RNA (copies/mL)			
≤6 months	738	404	872
>6 months	<80	160	264
Post ART endocervical HIV-1 RNA (copies/swab)			
≤6 months	<248*	3166	No samples available
>6 months	<248	<248	

Chapter 5. Antiretroviral therapy initiation is not associated with risky sexual behavior among heterosexual HIV-1 infected persons in serodiscordant partnerships

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**Antiretroviral therapy initiation is not associated with risky sexual behavior among heterosexual
HIV-1 infected persons in serodiscordant partnerships**

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ABSTRACT

Background: Few prospective studies have assessed whether antiretroviral therapy (ART) use is associated with changes in sexual risk behavior of HIV-1 infected persons in known HIV-1 serodiscordant partnerships.

Methods: We conducted a longitudinal analysis of 1817 HIV-1 infected persons with known uninfected partners enrolled in the Partners PrEP Study in Kenya and Uganda. We assessed the effect of ART on sexual risk behaviors using zero-inflated negative binomial regression.

Results: ART use was associated with a significant decrease in condomless sex acts with HIV-1 uninfected partners (rate ratio [RR] 0.64; 95% CI: 0.55-0.75; $p < 0.001$), but not condomless sex acts with non-primary partners (RR 0.94; 95% CI: 0.73-1.20; $p = 0.62$). Pregnancy incidence was lower after ART (HR 0.71; 95% CI: 0.60-0.84; $p < 0.001$). Incident STI diagnoses were similar (OR 1.05; 95% CI: 0.86-1.29; $p = 0.63$).

Conclusions: Substantial risk compensation did not occur following ART initiation among East African HIV-1 infected persons with known HIV-1 uninfected partners.

Introduction

Antiretroviral therapy (ART) reduces HIV-1 related morbidity and mortality and prevents HIV-1 transmission [4, 116]. ART could have the unintended consequence of increasing sexual risk behavior because of improved physical health and life expectancy, decreased perception of HIV-1 risk [34], and safe sex fatigue in stable partnerships. Risk compensation occurs when individuals who feel protected from a health risk engage in other risk behaviors because of changes in risk perception [33]. Risk perception may change because of knowledge that ART-mediated viral suppression reduces HIV-1 transmission risk (reduced-susceptibility optimism) [117-119], or the belief that ART significantly decreases HIV-1 related mortality (reduced-severity optimism) [120, 121]. Modelling studies suggest that the preventive benefits of expanded ART access could be offset by increased sexual risk behavior [35-37], because of risk compensation and/or prolonged survival, increasing lifetime duration of infectiousness. The success of ART for HIV-1 prevention is premised on regular HIV-1 testing, linkage of HIV-1 infected persons to care, ART initiation at any CD4 count, and the assumption that sexual risk behavior does not offset decreased HIV-1 infectiousness [34, 122].

Limited data are available regarding sexual risk behavior of ART exposed HIV-1 infected persons with known uninfected partners in sub-Saharan Africa. Prior studies of unprotected sex with serodiscordant or unknown status partners were cross-sectional and compared HIV-1 infected persons taking ART versus not, limited by small sample sizes, or had short duration of follow-up limiting assessment of the durable effect of ART on sexual risk behavior. We aimed to assess whether ART use is associated with increased frequency of high-risk sexual behavior in a large prospective study of HIV-1 serodiscordant couples in which the HIV-1 infected partner initiated ART.

Methods

Study setting and participants

Between 2008 and 2012, we conducted a prospective study of HIV-1 serodiscordant couples enrolled in the Partners PrEP Study, a randomized clinical trial of antiretroviral pre-exposure prophylaxis in Kenya and Uganda [53]. At baseline, HIV-1 infected partners were sexually active and ART naive. During follow

up, they received clinical and immunological monitoring for ART eligibility, and referrals to partnering HIV-1 care organizations when eligible for treatment. At each quarterly study visit, data on ART use and sexual behavior within the primary partnership and with other partners was collected using semi-structured questionnaires. During the study period, national treatment guidelines changed from recommending ART for symptomatic HIV-1 disease or CD4 count <250 cells/ μ L to CD4 count \leq 350 cells/ μ L, in line with evolving World Health Organization guidance. All study participants received HIV-1 prevention services including individual and couple risk-reduction counseling, free condoms, and screening and treatment of sexually transmitted infections.

Institutional review boards at the University of Washington and collaborating institutions at each of the study sites approved the study protocol. All participants provided written informed consent in English or their local language.

Laboratory methods

Urine pregnancy testing for HIV-1 uninfected women partners was performed monthly. Testing for sexually transmitted infections (STIs) was conducted annually and when clinically indicated. Syphilis serology was performed using rapid plasma reagin tests and positive results confirmed using *Treponema pallidum* particle agglutination tests. We tested for for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using APTIMA Combo 2 (Gen-Probe) or COBAS Amplicor (Roche Diagnostic) assays and for *Trichomonas vaginalis* using APTIMA TV TMA (Gen-Probe) or In Pouch TV (Biomed Diagnostics).

Statistical analysis

The primary outcomes were count of condomless sex acts (within and outside the primary partnership) and, as markers of condomless sex, STI diagnoses and pregnancy incidence (including pregnancies in both HIV-1 infected and uninfected women to capture the partnership nature of the data). Sexual activity was assessed for the prior month, at each quarterly visit. The distribution of sex acts was positively skewed, overdispersed relative to the Poisson distribution (i.e., conditional variance larger than the conditional mean), and zero-inflated. Zero inflation was due to an excess number of zero sex acts, compared to a normal Poisson distribution, which occurred because of abstinence, partnership

dissolution or perfect condom use. We evaluated Poisson, negative binomial, and zero-inflated Poisson and negative binomial regression models for best fit. Vuong tests suggested that zero-inflated models provided a better fit than standard regression models [123]. Akaike Information Criteria (AIC) and Bayesian Information Criteria (BIC) indicated that the zero-inflated negative binomial regression (ZINB) model fit the data better than the zero-inflated Poisson model. The ZINB model combines a negative binomial model (for overdispersed count data) and a logistic model (to assess zero inflation). Thus, we report two exponentiated regression coefficients for the association of ART use with sexual behavior: rate ratios from the negative binomial model (for the proportional change in condomless sex acts after ART) and odds ratios from the logistic model (for the relationship between ART use and odds of abstinence, partnership dissolution or perfect condom use). The negative binomial and logistic models were not fit with identical covariates. Potential confounders which changed the beta coefficient for ART by more than 10% were included in adjusted models. We performed sub-group analyses in women, men and younger HIV-1 infected persons (individuals <30 years may be more likely to desire children). We used robust standard errors in all models to account for within-subject correlation. Pregnancy incidence was compared using the Andersen-Gill extension of the Cox regression model, to account for multiple pregnancies per woman. A binary STI variable was created to indicate diagnosis with gonorrhea, chlamydia, trichomonas or syphilis infection; STI incidence was evaluated using logistic regression with generalized estimating equations and robust standard errors to account for multiple observations per person. Statistical analyses were performed using Stata 12.1 (StataCorp, College Station, TX) and SAS version 9.4 (SAS Institute, Cary, NC).

Results

Participant characteristics

Of 4747 HIV-1 infected women and men enrolled, 1817 initiated ART during the study, of which 1062 (58%) were women. At enrollment, the median age of HIV-1 infected men and women was 40 years (interquartile range [IQR] 35-45), and 30 years (IQR 25-36), respectively (Table 7). Most (98%) were married and reported a median of 4 sex acts (IQR 2-8) with the primary partner in the prior month. HIV-1 infected men had longer duration of partnership (13 vs. 6 years; $p < 0.001$) and were less likely to report

condomless sex in the prior month (21 vs. 29%; $p=0.01$). Nine percent of HIV-1 infected women were pregnant, and 6% of HIV-1 infected men reported condomless sex with other partners. *Trichomonas vaginalis* was the most prevalent STI and was identified in 10% of women and 2% of men.

Sexual behavior and ART

The 1817 HIV-1 infected women and men contributed 10,369 person months of observation before ART initiation and 9,255 person-months after ART. The median CD4 and plasma HIV-1 RNA concentration at ART initiation were 277 cells/ μ L (IQR 220-355) and 4.18 \log_{10} copies/mL (IQR 3.53-4.73). We observed significant reductions in condomless sex acts with primary partners (4.40 versus 3.12 per month; RR 0.64; 95% CI: 0.55-0.75; $p<0.001$) [Table 8; negative binomial model]. This decline in condomless sex acts was observed in serodiscordant couples in which the HIV-1 infected partner was female (RR 0.61; 95% CI: 0.51-0.74; $p<0.001$), male (RR 0.68; 95% CI: 0.52-0.89; $p=0.006$) and <30 years (RR 0.57; 95% CI: 0.41-0.79; $p=0.001$). Overall, ART use was associated with lower odds of abstinence, partnership dissolution or perfect condom use (adjusted odds ratio [AOR] 0.75; 95% CI: 0.60-0.94; $p=0.01$) [Table 8; logistic model]. In non-primary partnerships, we observed a non-significant decline in the number of condomless sex acts (4.04 versus 3.49 per month; RR 0.94; 95% CI: 0.73-1.20; $p=0.62$), and a trend towards greater odds of abstinence, partnership dissolution or consistent condom use (AOR 1.35; 95% CI: 0.96-1.91; $p=0.09$).

Pregnancy and STI incidence

The median time from ART initiation to first pregnancy was 7.8 months (IQR 2.7-13.8). The pregnancy incidence rate declined from 13.2 per 100 woman-years (1215 pregnancies; 9191 woman-years) prior to ART initiation to 8.4 per 100 woman-years (174 pregnancies; 2078 woman-years) after ART (HR 0.71; 95% CI: 0.60-0.84; $p<0.001$). Pregnancy incidence decreased in serodiscordant couples with HIV-1 uninfected women (11.2 vs. 6.4 per 100 woman-years; HR 0.68; 95% CI: 0.50-0.92; $p=0.01$) and HIV-1 infected women (14.5 vs. 9.8 per 100 woman-years; HR 0.76; 0.62-0.93; $p=0.006$). We diagnosed 462 incident STIs at 5169 visits, including 272 (59%) cases of *Trichomonas*, 99 (21%) cases of gonorrhea and

74 (16%) cases of syphilis infection. No significant differences in incident STI diagnoses were observed (8.3 vs. 8.7% of visits; OR 1.05; 95% CI: 0.86-1.29; p=0.63).

Discussion

In this prospective study, that included approximately 1800 East African heterosexual HIV-1 infected persons with known HIV-1 uninfected partners, ART use was associated with significant reductions in condomless sex acts with primary partners. This decrease in condomless sex acts was observed in HIV-1 infected woman and men and younger HIV-1 infected persons. ART use was also associated with decreased pregnancy incidence, regardless of the woman's HIV-1 status. There was neither a significant increase nor decrease in self-reported condomless sex with non-primary partners or STI incidence.

Our finding that HIV-1 infected women and men were significantly less likely to self-report condomless sex with primary partners after starting ART is similar to previous reports [13, 106, 124-132]. A meta-analysis of 14 studies of sexual behavior and ART use in sub-Saharan Africa [133] identified only four studies [126, 127, 129, 131] that reported rates of unprotected sex with HIV-1 uninfected or unknown status partners. ART use was associated with a reduction in unprotected sex (OR 0.55; 95% CI: 0.30-0.99; p<0.001) but heterogeneity was significant ($I^2=85.2\%$). In another meta-analysis, ART use was associated with decreased unprotected sex with HIV-1 uninfected or unknown status partners (OR 0.64: 95% CI: 0.46-0.88, p<0.001; heterogeneity $I^2=61\%$) [134]. Our results show that ART use was associated with a lower likelihood of sex unprotected by condoms irrespective of the gender of the HIV-1 infected partner. A study from the same setting reported decreased sexual risk behavior in women (OR 0.85 per year of ART) but not men (OR 1.41 per year of ART) [132]. The decreased odds of abstinence we observed suggest that the reduction in condomless sex was due to increased condom use with primary partners.

We did not observe significant reductions in condomless sex acts with non-primary partners after ART initiation. Prior work in this setting suggests differences in HIV-1 risk perception lead to risk shifting from the known serodiscordant partnership to outside partners, perhaps due to relationship dissolution and

new partnership formation [135]. HIV-1 uninfected persons in this cohort reported decreased sexual frequency in the primary partnership and increased sexual activity with non-primary partners [136]. Notably, condomless sex was frequent in the non-primary partnerships. In contrast, HIV-1 infected women and men did not report increased condomless sex with non-primary partners in this same cohort. These data suggest no evidence of behavioral risk compensation in HIV-1 infected persons following ART initiation irrespective of partnership type.

Pregnancy incidence decreased by 32% among serodiscordant couples with HIV-1 uninfected women and 24% among couples with HIV-1 infected women. This decline corroborates the observed decrease in self-reported condomless sex. Decreased pregnancy incidence following ART initiation has been reported in the same setting, but occurred after 24 months of ART [112]. In this study, there was no association between self-reported male partner HIV-1 status and pregnancy incidence. In contrast, a large study of 4531 HIV-1 infected women in 7 sub-Saharan African countries reported a 74% increase in pregnancy incidence after ART [137], as have other studies in the same setting [138, 139]. We did not observe significant differences in STI incidence in HIV-1 infected persons or their HIV-1 uninfected partners [136], likely because of low STI prevalence in this population of married heterosexual couples.

The strengths of our study include the serodiscordant couple design permitting within-couple analyses, the large sample size providing statistical power to evaluate within-subject comparisons (pre-ART versus post-ART behavior) which are less subject to confounding than comparisons of ART-exposed versus non-exposed persons, and the use of pregnancy and STI incidence as objective markers of condomless sex. Our study has limitations. HIV-1 serodiscordant couples received regular risk reduction counseling which may have reduced the likelihood of risky behavior. Self-report of sexual behavior is subject to recall bias or social desirability, but this was validated by biologic measures of unprotected sex. We tested for bacterial STIs annually or when clinically indicated and may have underestimated the true rate of STIs in this population. The short duration of follow up after ART initiation may have limited our ability to detect long-term trends in sexual behavior. Nevertheless, studies with longer duration of follow up have reported similar outcomes.

In conclusion, we did not find evidence of increased sexual risk behavior in mutually-disclosed HIV-1 serodiscordant couples which received counseling about ART prevention benefits. These data support the importance of ART programs offering counseling about ART and HIV-1 transmission risk.

Author contributions

AM and JMB designed the study. AM and JMB wrote the first draft. AM performed the statistical analyses. All authors contributed to data collection, interpretation of the results and the writing of the manuscript, and all approved the final draft.

Competing interests

The authors report no competing interests.

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Ethical approval: “All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.”

Informed consent: “Informed consent was obtained from all individual participants included in the study.”

Table 7: Enrollment characteristics of HIV-infected women and men initiating ART

	HIV-infected women (N=1062)	HIV-infected men (N=755)
Median (IQR) or N (%)		
Demographic characteristics		
Age (years)	30 (25-36)	40 (35-45)
Education (years)	7 (4-7)	7 (4-10)
Monthly income (any)	604 (57)	641 (85)
Couple characteristics		
Married to HIV-uninfected study partner	1033 (97)	748 (99)
Duration of partnership (years)	6 (3-11)	13 (6-19)
Number of children with study partner	2 (0-3)	3 (2-5)
Number of sex acts with study partner, prior month	4 (3-8)	4 (2-6)
Any unprotected sex acts with study partner, prior month	303 (29)	162 (21)
Multiple partners	8 (1)	115 (15)
Any unprotected sex acts with other partners, prior month	2 (<1)	43 (6)
Pregnant (women only)	93 (9)	...
Sexually Transmitted Pathogen		
<i>Trichomonas vaginalis</i>	106 (10)	14 (2)
<i>Neisseria gonorrhoeae</i>	13 (1)	3 (<1)
<i>Chlamydia trachomatis</i>	10 (1)	0 (0)
<i>Treponema pallidum</i>	32 (3)	37 (5)

Table 8. Sexual risk behaviors and associations with ART

Variable	Mean sex acts per month (variance)		Negative Binomial Model ^a		Logistic Model	
	Before ART	After ART	RR* (95% CI)	P	OR** (95% CI)	P
Study partner						
Sex acts	4.40 (21.50)	3.12 (13.91)	0.82 (0.79-0.86)	<0.001	2.89 (2.41-3.46) ^b	<0.001
Condomless sex acts	0.65 (5.72)	0.39 (3.09)	0.64 (0.55-0.75)	<0.001	0.75 (0.60-0.94) ^c	0.01
HIV-infected women	0.79 (7.40)	0.48 (4.14)	0.61 (0.51-0.74)	<0.001	0.81 (0.60-1.11)	0.20
HIV-infected men	0.46 (3.19)	0.27 (1.66)	0.68 (0.52-0.89)	0.006	0.80 (0.56-1.16)	0.24
Age <30 years	0.88 (8.26)	0.52 (4.23)	0.57 (0.41-0.79)	0.001	0.59 (0.30-1.14)	0.12
Other partners						
Sex acts ^d	4.04 (14.14)	3.49 (10.28)	0.98 (0.83-1.14) ^e	0.77
Condomless sex acts	1.30 (9.23)	1.04 (6.88)	0.94 (0.73-1.20)	0.62	1.35 (0.96-1.91)	0.09
HIV-infected women	1.22 (4.54)	1.15 (6.84)	1.24 (0.77-2.00)	0.38	1.71 (0.62-4.69)	0.30
HIV-infected men	1.32 (10.20)	1.00 (6.89)	0.84 (0.62-1.13)	0.25	1.28 (0.88-1.88)	0.20
Age <30 years	1.63 (15.22)	1.29 (7.44)	0.64 (0.20-2.02)	0.44	1.09 (0.12-10.7)	0.94

^aall outcomes adjusted for total sex acts or condomless sex acts at enrollment.

^badjusted for age and gender.

^cadjusted for time-varying age.

^dnegative binomial model (by study design, only non-zero sex acts with other partners were documented).

^eadjusted for gender.

*rate ratios represent the proportional change in condomless sex acts with ART use.

**odds ratios represent the relationship between ART use and odds of abstinence, partnership dissolution or perfect condom use.

Chapter 6. Discussion

The analyses presented here confirm that ART decreases HIV-1 infectiousness, by suppressing HIV-1 RNA concentrations in blood and genital secretions. There was residual risk of HIV-1 transmission prior to complete suppression of HIV-1 RNA concentrations in blood plasma and genital secretions, and no HIV-1 transmission events on suppressive ART. Younger age independently predicted failure to achieve viral suppression and virologic rebound after initial suppression. We did not find evidence of increased sexual risk behavior immediately following ART initiation. The results of this dissertation enrich our understanding of the biologic preventive effect of ART in decreasing HIV-1 infectiousness and transmission risk. As countries move towards adopting WHO guidelines recommending earlier treatment, future research should evaluate interventions to maximize the potential of ART for HIV-1 treatment and prevention.

Interpretation of findings

Chapter 2: Younger age predicts poor virologic outcomes.

Evidence-based interventions to improve ART adherence in younger adults are urgently needed. In chapter 2, we found high levels of viral suppression and low rates of virologic rebound suggesting HIV-1 infected partners in serodiscordant partnerships were motivated to take ART, for their own health and to prevent HIV-1 transmission. Younger age was associated with failure to achieve plasma viral suppression and virologic rebound after initial suppression. Younger age is associated with delayed ART initiation [51], lower rates of HIV-1 virologic suppression [56, 57], virologic rebound [68], low PrEP adherence [61], and lack of protection from HIV-1 in vaginal dapivirine ring trials [140, 141]. This consistent finding across multiple studies and settings is likely due to the correlation between younger age and nonadherence [142-144].

Our results highlight the need for ART programs to address nonadherence in younger persons accessing antiretrovirals for HIV-1 treatment and prevention. Adolescence and young adulthood is a period of physical, cognitive, emotional and social change, in which risk-taking behaviors are biologically driven, and result from changes in the socio-emotional system of the brain [145]. These include dramatic

changes in dopaminergic activity in the brain (dopaminergic remodeling), which is more pronounced in boys [145, 146]. Gradual maturation of the pre-frontal cortex improves an individual's ability to weigh risks and benefits, self-regulate impulsive behavior, and plan long-term. This maturation process is typically completed in the mid-20's, and results in reduced risk taking [145]. Thus, nonadherence may be a behavioral manifestation of developmental neurobiological changes in the brain.

Evidence-informed interventions to improve ART adherence in younger adults should address programmatic and patient-level barriers, including low risk perception due to feelings of invulnerability, denial of HIV-1 diagnosis, stigma, lack of disclosure, fear of side effects, substance use, depression and multiple pre-ART counseling visits [45-49]. Removing programmatic barriers to ART initiation, by decentralizing, task shifting and integrating HIV-1 services into primary care, increases treatment coverage [147]. Patient-level interventions include once-daily regimens, fixed dose combinations, reminder devices, provision of one-on-one or couple-based adherence support with feedback about viral suppression and mobile health technology [88, 89]. Mobile phone use is widespread in sub-Saharan Africa, and mobile health technology (mHealth) is efficacious in enhancing ART adherence and improving viral load suppression [148]. mHealth could improve treatment outcomes in younger HIV-1 infected persons (female sex workers, truckers, migrant workers, fisher folk) who have mobile lifestyles and are difficult to retain in care [147]. In a randomized trial of daily mobile phone adherence support, youth aged 15-24 years who received medication reminders had better adherence and lower viral loads than those who did not receive reminders [149]. Short message service (SMS) improves engagement and retention in HIV-1 care, and ART adherence in sub-Saharan Africa [150]. ART programs should consider using automated text messaging and messaging applications to support ART adherence in youth. Future studies should conduct operational research to determine programmatic determinants of adherence and retention in younger persons and evaluate their comparative effectiveness. Implementation scientists should evaluate the feasibility and cost effectiveness of integrating mHealth into social media applications (e.g. WhatsApp), including for young, stigmatized and hard-to-reach populations.

Chapter 3: Seminal HIV-1 RNA detection on ART

In chapter 3, we described the largest study of seminal HIV-1 RNA suppression in sub-Saharan Africa. Following a considerable logistical effort at nine research sites in two countries, nearly 500 ART initiators provided semen samples. We found that seminal HIV-1 RNA detection was infrequent and low quantity in African heterosexual men initiating ART. The majority of men who shed HIV-1 in semen had RNA concentrations <1000 copies/mL, a threshold below which HIV-1 transmission is rare [7]. Seminal HIV-1 shedding was strongly associated with detection of HIV-1 RNA in blood plasma.

Universal ART coverage is essential for ending the AIDS epidemic. Men account for nearly half of the 26 million people living with HIV-1 in sub-Saharan Africa, but only 36% of those receiving treatment [151]. HIV-1 prevention and care programs have traditionally focused on women and children, with few targeted interventions for men [152]. Early linkage to HIV-1 care and ART initiation decreases HIV-1 transmission [153], but men are significantly less likely than women to initiate ART when eligible, in a setting where pregnancy is a significant determinant of ART access [154]. Male sex predicts poor retention in HIV-1 care in sub-Saharan Africa, in part because of migratory labor patterns of fishermen, agricultural workers, and truckers [155]. Targeted HIV-1 prevention interventions have had mixed results because male health seeking behavior is poorly understood [156].

Men face significant barriers accessing HIV-1 care. These include stigma, difficulties accessing health care during working hours, and prioritization of maternal and child health services in HIV-1 prevention programs, factors which exacerbate gender inequities in ART access [157]. Cultural constructs of masculinity perceive ill health as weakness and lead to poor health seeking behavior while simultaneously encouraging high-risk behaviors as expressions of manhood. These include multiple sexual partnerships, preference for condomless sex, violence against women, and alcohol and substance abuse [152, 158]. Evidence-informed interventions to improve early HIV-1 diagnosis and treatment include integrating vertical medical male circumcision, TB, and ART programs [159, 160], decentralization and task shifting [161], home HIV-1 testing and ART initiation [162], peer support [163], and workplace programs [164]. Placing HIV-1 services in bars, nightclubs, prisons, and military, law enforcement and sports facilities increases male access [165]. Health systems should adopt data-driven approaches that

emphasize male participation and involvement in HIV-1 prevention and care. Men should be enabled to transfer HIV-1 care to other treatment facilities when they migrate. Future studies should develop targeted culturally appropriate interventions that address masculinity and sexual health to improve uptake of HIV-1 services. International, national and donor policies should emphasize the importance of engaging men early in HIV-1 care [166], avoid blaming them for spreading HIV-1, and simplify and decentralize ART delivery for working men. In order to achieve UNAIDS 90-90-90 targets, targeted and sustainable funding of HIV-1 services for men are needed.

Chapter 4: HIV-1 transmission risk on ART

In chapter 4, we found residual risk of HIV-1 transmission during the first six months of ART, with three phylogenetically linked female-to-male transmissions occurring soon after the female partner reported ART initiation, when HIV-1 RNA concentrations in blood and genital secretions were still detectable. There were no HIV-1 transmission events on suppressive ART despite self-report of condomless sex and high pregnancy incidence.

The population-level effectiveness of ART for HIV-1 prevention is dependent on high coverage. The Joint United Nations Program on HIV/AIDS (UNAIDS) 90-90-90 targets states that by 2020, 90% of HIV-1 infected people should know their HIV status, 90% of persons with known HIV-1 infection will receive ART, and 90% of all ART recipients will achieve viral suppression [167]. However, a majority of people (67% of men and 57% of women) known to be living with HIV-1 in sub-Saharan Africa were not receiving ART in 2013 [168]. Effective scale-up of treatment in sub-Saharan Africa requires translation of emerging evidence into policy, programs and practices. The HIV-1 prevention impact of early ART will be maximized if testing and treatment are made available to high-risk populations. Community approaches (door-to-door, multi-disease prevention campaigns, peer outreach, mHealth) increase coverage of HIV-1 testing, linkage to care and awareness of the prevention benefits of ART [169]. Several African countries have begun to implement 'test and treat' policies for key populations in which the HIV-1 epidemic is concentrated. These include serodiscordant couples (Malawi, Uganda, Tanzania, Zambia), sex workers (Rwanda, Uganda, Zambia), people who inject drugs (Malawi, Tanzania), men who have sex with men

(Tanzania, Zambia), fisherfolk (Uganda) and truckers (Uganda) [147]. As countries expand treatment access to meet UNAIDS 90-90-90 targets, lessons learned from key populations will inform ART scale up to the entire population of people living with HIV-1. Increased ART coverage reduces the proportion of ART naïve persons initiating treatment with advanced HIV-1 disease and decreases morbidity and mortality and HIV-1 incidence. Implementation of evidence-informed prevention interventions, including condoms and PrEP, complements the preventive benefits of ART, particularly during the period prior to complete viral suppression. An open-label prospective study showed that offering PrEP to the HIV-1 uninfected partner until the HIV-1 infected partner had achieved viral suppression (“PrEP as a bridge to ART”) nearly eliminated HIV-1 transmission [115]. Integrated delivery of ART and PrEP for HIV-1 serodiscordant couples in sub-Saharan Africa will further decrease HIV-1 incidence. Health systems strengthening should focus on streamlining regulatory approval, procurement and distribution of generic, low cost, and fixed-dose combination antiretroviral drugs. HIV-1 care should be integrated into primary health care and delivered alongside general health, maternal and child, TB, and STI services. Implementation of decentralized ART delivery, and task shifting to nurses, midwives, clinical officers and community health workers should be prioritized. Point-of-care viral load testing is urgently needed in primary care settings. National programs should invest in strong monitoring and evaluation systems to track the continuum of HIV-1 care and institute corrective action in a timely manner. Sub-Saharan countries will need external donor support to meet the considerable cost of universal ART and to reduce delays in adopting and implementing rapidly changing treatment guidelines.

Chapter 5: Sexual risk behavior before and after ART

In Chapter 5, we found significant decreases in condomless sex and pregnancy incidence in the primary partnership after ART initiation. However, STI incidence and sexual risk behavior in non-primary partnerships were not significantly different. This study provides further evidence that the preventive benefits of suppressive ART described in Chapters 2, 3 and 4, do not appear to be offset by increased sexual risk behavior soon after treatment initiation.

A growing body of evidence suggests it may be time to put the risk compensation theory to rest [170].

Despite concerns that ART use could increase sexual risk-taking, several studies from a variety of settings have found no evidence of increased sexual risk behavior following ART initiation as measured by self-report of condomless sex and STI incidence, regardless of gender, sexual practices or geographical location [134]. These reductions in condomless sex have been observed in key populations including serodiscordant partnerships, injecting drug users and female sex workers, and may be due to the behavioral effect of regular risk reduction counseling and condom provision. Notably, 11 studies of MSM found no effect of ART on self-report of unprotected sex [134]. The observed decrease in sexual risk behavior coupled with decreased HIV-1 infectiousness on suppressive ART previously described, underscores the potential of ART for ending the AIDS epidemic. Risky sexual behaviors (condomless sex, multiple sexual partners) and poor medication adherence are associated with alcohol consumption in sub-Saharan Africa [171]. In this setting, alcohol consumption symbolizes masculinity and socioeconomic status. Although alcohol abuse is a modifiable risk factor, it is rarely addressed in health policy or HIV-1 prevention programs. Sub-Saharan Africa has the highest prevalence of HIV-1 and heavy episodic drinking in the world, but few interventions targeting alcohol and HIV-1 risk have been developed or implemented in this region [172]. Couple-based interventions focusing on alcohol-using men and their sexual partners reduce heavy drinking, increase condom use, and decrease HIV incidence [173]. Implementation of structural interventions to reduce alcohol abuse in this setting should address the widespread consumption of homemade alcohol, restricted alcohol sales to intoxicated persons, sale of alcohol to minors, and driving under the influence. Future studies should develop and test culturally appropriate interventions targeting alcohol-related sexual risk behaviors in HIV-1 infected women and men in sub-Saharan Africa.

STI diagnosis and treatment services are an important component of combination HIV-1 prevention. Although we found no association between ART use and STI incidence, gonorrhea, chlamydia, trichomonosis and syphilis exact a heavy toll in sub-Saharan Africa [174]. Undiagnosed and untreated STIs have a disproportionate impact on young people, women and sexual minorities in this setting, because diagnostics are not routinely available or performed in HIV-1 care facilities, and treatment services are underutilized. Future studies should evaluate feasibility and cost-effectiveness of point-of-

care diagnostics to facilitate prompt STI diagnosis, treatment and partner services [175].

Conclusion

HIV-1 serodiscordant couples are a unique population in which to study HIV-1 infectiousness and transmission risk. The prospective studies presented here utilized high quality behavioral, clinical, and laboratory data from HIV-1 infected and uninfected partners to demonstrate the secondary prevention benefits of HIV-1 treatment. We used self-reported sexual behavior, incidence of pregnancy and sexually transmitted infections, viral suppression in blood and genital secretions, and phylogenetic linkage of HIV-1 transmission events to evaluate HIV-1 transmission risk. We showed that there was residual HIV-1 transmission risk prior to complete viral suppression. We did not observe any transmission events after six months of suppressive ART. Importantly, this biologic preventive effect of ART was not offset by increased sexual risk behavior in HIV-1 infected women and men initiating ART. ART is the most potent biomedical HIV-1 prevention intervention and substantially decreases HIV-1 incidence at the population level. Expanding HIV treatment results in economic benefits because of averted healthcare costs, averted orphan care and gains in labor productivity. Integration and high coverage of evidence-based behavioral, biomedical and structural interventions in key populations and settings will maximize the effectiveness of ART for HIV-1 prevention and hasten the end of the AIDS epidemic.

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