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Unreported antiretroviral use by HIV-1 infected participants enrolling in a prospective research study

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The concentration of viral RNA in plasma is the primary risk factor for sexual transmission of HIV-1 [1–3], and reductions in plasma HIV-1 RNA levels due to antiretroviral therapy (ART) result in marked decreases in HIV-1 transmission risk [4,5]. Results from studies of HIV-1 transmission and disease progression may be more difficult to interpret if a substantial proportion of HIV-1 infected partners have low or undetectable viral loads on ART, and thus, ART use at study enrollment is often an exclusion factor.

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Recent reports from clinical trial cohorts of HIV-1 transmission in HIV-1 serodiscordant couples have found that nearly a quarter of HIV-1 infected partners had low enrollment plasma HIV-1 RNA levels (<2000 copies/ml) [6,7]. Low levels of plasma HIV-1 RNA in the HIV-1 infected partners, selected for not having transmitted HIV-1 to their partner for studies of candidate interventions to reduce HIV-1 transmission, may reflect natural host control of viral replication. However, an alternative explanation could be unreported ART use. Distinguishing between these potential sources of low viral load is important for studies seeking to understand the biology of HIV-1 transmission. We tested stored samples from a recent HIV-1 prevention clinical trial to determine the frequency of unreported ART use among HIV-1 infected individuals with low plasma HIV-1 RNA levels.

Between November 2004 and April 2007, we enrolled 3408 heterosexual HIV-1 serodiscordant couples from seven African countries in a randomized, double-blind, placebo-controlled clinical trial of herpes simplex virus type 2 (HSV-2) suppressive therapy to reduce HIV-1 transmission (Partners in Prevention HSV/HIV Transmission Study), as previously described [6]. Eligible couples were at least 18 years of age, sexually active, and intending to remain as a couple. All HIV-1 infected partners were HSV-2 seropositive, had CD4 counts ≥ 250 cells/ μ L (making them ineligible for antiretroviral therapy under national guidelines of the study countries at that time), not pregnant, and self-reported not currently taking ART. Quarterly plasma and serum samples were collected for up to 24 months and archived at -80°C for subsequent laboratory testing. HSV-2 suppressive therapy did not reduce HIV-1 transmission within the study partnerships [8]. At study screening and all follow-up visits, HIV-1 infected participants were asked if they were currently taking ART.

All participants received HIV-1 primary care, referral for ART according to national guidelines and risk reduction counseling and treatment for sexually transmitted infections during up to 24 months of study follow-up. Written, informed consent was obtained from all participants. The study protocol was approved by the University of Washington Human Subjects Review Committee and ethical review committees at each of the study sites.

All laboratory testing occurred at the end of study follow-up. Plasma HIV-1 RNA levels were quantified using the COBAS Ampliprep/COBAS TaqMan real-time HIV-1 RNA assay, version 1.0 (Roche Diagnostics, Indianapolis, IN), with a lower limit of quantification of 240 copies/mL. For those with plasma HIV-1 RNA <2000 copies/mL at enrollment, high-performance liquid chromatography with ultraviolet detection was used to measure antiretroviral (ART) levels in an archived serum collected at enrollment [9]. The assay used to measure ART levels was validated for five nucleoside reverse transcriptase inhibitors (abacavir, didanosine, lamivudine, stavudine and zidovudine) and one non-nucleoside reverse transcriptase inhibitor (nevirapine). We considered any quantifiable concentration as indicative of ART use.

Low viral load was stratified into two groups defined as: 1) low detectable (240–2000 copies/mL), or 2) undetectable (<240 copies/mL). We calculated the overall prevalence of unreported ART use at enrollment among HIV-1 infected partners with low plasma HIV-1 RNA (<2000 copies/ml) overall and in each group. All analyses were conducted using SAS (v.9.2, Cary, NC).

Among 3371 HIV-1 infected partners who had results for enrollment plasma HIV-1 RNA tested, 798 (23.7%) had plasma HIV-1 RNA <2000 copies/mL, including 443/798 (13.1%) with low plasma HIV-1 RNA levels (240–2000 copies/mL) and 355/798 (10.5%) with undetectable RNA (<240 copies/mL). Those with enrollment plasma HIV-1 RNA <2000 copies/mL were more likely to be female compared to those with plasma HIV-1 levels

>2000 copies/mL (78.1% vs. 64.1%, $p<0.05$); all other characteristics were similar for those with higher versus lower plasma HIV-1 RNA (Table).

ART testing was performed on 771 (96.6%) of the persons with plasma HIV-1 RNA <2000 copies/mL where specimens were available. Antiretrovirals were detected in 171/771 (22.2%): 157/341 (46.0%) in those with undetectable plasma HIV-1 RNA (<240 copies/mL) and 14/430 (3.3%) in those with low detectable plasma HIV-1 RNA (240–2000 copies/mL). The most common ARTs detected were lamivudine (20.8%) and nevirapine (17.8%). Most, (83.6%), of the 171 participants with detectable ARTs had evidence of multiple drugs, specifically the combinations nevirapine/lamivudine (52.0%), nevirapine/lamivudine/stavudine (21.6%), stavudine/lamivudine (5.3%), and zidovudine/lamivudine with or without nevirapine (4.1%). Differences in ART detection were found among the study sites, but there were no difference in ART detection between men and women.

We have previously reported that nearly a quarter (23.7%) of HIV-1 infected partners in HIV-1 serodiscordant partnerships for the Partners in Prevention HSV/HIV Transmission Study had plasma HIV-1 levels <2000 copies/mL at baseline, in the absence of reported use of ART [6]. Our analysis here demonstrates that 22% of those, and nearly half of the subset with undetectable plasma HIV-1, had evidence of unreported ART use. Thus, undetectable plasma HIV-1 RNA is a potential marker of unreported ART use in HIV-1 infected partners. This finding could be significant for studies focused on describing host factors associated with natural viral control, since a large proportion of individuals with low plasma HIV-1 levels had pharmacologic and not immunologically induced viral suppression. For example, in genetic studies of elite controllers, the inclusion of subjects with ART-induced viral suppression would undermine the ability of the study to identify any potentially valuable genetic markers.

It is important to note that while unreported ARV detection was strongly associated with plasma HIV-1 level (48% for undetectable versus only 3% for detectable but <2000 copies/mL), the proportion of individuals in the overall study cohort with unreported ART use and detectable ARVs was very small (171/3408, 5%) and equally distributed between the randomization arms in the clinical trial. For these reasons, it is unlikely that unreported ART use would have an important impact on the overall outcomes of this clinical trial, or other randomized clinical trials of this kind.

Since ART use was an exclusion criterion in this study and thus by definition was not reported, inferences about reasons or circumstances underlying this finding are largely speculative. Women had a significantly higher proportion of plasma HIV-1 RNA <2000, suggesting women may have been more likely to have received ART, possibly clinically indicated for prevention of mother-to-child transmission (PMTCT) for which nevirapine and lamivudine are included in recommended regimens and also prominently represented among the ARVs detected in this analysis [10–11]. However, in our analysis, we did not find significant difference in ART by gender and do not have evidence that women were more likely to have unreported ART detected. Without knowing specific timing of recent doses, we cannot make any determination on whether or not persons with detected ARV discontinued drug use prior to study screening or what drug dose was taken. Nevirapine can be detected in women more than two weeks after receiving single-dose nevirapine for PMTCT [12–13], while most nucleoside reverse transcriptase inhibitors such as lamivudine and stavudine can be detected, at most, for a few days after discontinuation [14].

In resource-limited communities, services and other benefits offered through clinical trial participation could provide an incentive to not disclose ART use, which would have made them ineligible for the trial [15]. Couples enrolled in the Partners in Prevention HSV/HIV

Transmission Study did not receive a monetary incentive for participation but did receive benefits including free counseling, screening and treatment for sexually transmitted infections, condoms and travel reimbursement. One prior study of participation in a large-scale HIV-1 prevention trial in South Africa concluded that the level of reimbursement could be a motivating factor for some participants to misreport information during enrollment screening [16]. In multiple studies of willingness to participate in HIV-1 clinical trials in different settings, the majority of participants reported primarily altruistic motivations for participants, although a minority or respondents stated monetary incentives and access to health care as the primary motivator for participation [17–19]. We recommend further research to understand nondisclosure of ART by participants enrolling in an HIV-1 prevention study.

In summary, we found unreported use of ART to be prevalent among HIV-1 infected individuals with undetectable plasma virus. For randomized control trials of HIV-1 prevention interventions in HIV-1 serodiscordant couples or of novel HIV-1 treatments, assessing viral load may improve the efficiency of the study, by excluding those with low viral loads who would be unlikely to transmit or have a substantial virologic response to treatment. More importantly, for observational studies of pathogenesis and transmission, it may be critical to understand the etiology of undetectable viral loads, and thus particularly important to identify unreported ART use. Studies recruiting HIV-1 infected participants with low levels of plasma HIV-1 RNA, should consider laboratory testing for ART.

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Table

Enrollment characteristics and type of antiretroviral (ART) detected among HIV-1 infected partners

	2000 copies/mL, N=2573	<2000 copies/mL, N=443	Undetectable, N=355
Demographic characteristics			
Female gender	1650 (64.1%)	335 (75.6%)	288 (81.1%)
Age, years	32 (27–39)	31 (26–37)	32 (28–37)
Education, years	8 (6–11)	8 (7–12)	8 (6–11)
Has monthly income	940 (36.3%)	159 (35.9%)	120 (33.8%)
Couple characteristics			
Married and/or cohabiting	2340 (90.9%)	399 (90.1%)	324 (91.3%)
Duration of partnership, years	5.3 (2.3–10.4)	4.8 (2.2–9.6)	6.2 (2.6–10.6)
Number of children	1 (0–2)	1 (0–2)	1 (0–3)
Sex with outside partner, prior 30 days	92 (3.6%)	16 (3.6%)	7 (2.0%)
Any unprotected sex, prior 30 days	725 (28.2%)	120 (27.1%)	117 (33.0%)
Clinical characteristics			
CD4 count, cells/mm ³	458 (345–626)	471 (352–640)	467 (348–650)
Any sexually transmitted infection*	380 (14.8%)	56 (12.6%)	51 (14.4%)
Antiretroviral (ART) tested		N=430	N=341
NRTIs			
Abacavir (ABC)		0 (0%)	1 (0.3%)
Zidovudine (AZT)		0 (0%)	9 (2.6%)
Lamivudine (3TC)		12 (2.8%)	148 (43.4%)
Stavudine (D4T)		3 (0.7%)	43 (12.6%)
Didanosine (DDI)		0 (0%)	1 (0.3%)
NNRTIs			
Nevirapine (NVP)		11 (2.6%)	126 (37.0%)
Any ART detected		14 (3.3%)	157 (46.0%)

* Including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*