The Journal of Infectious Diseases

BRIEF REPORT

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

Andrew Mujugira,[1,2] Robert W. Coombs,[3,4] Renee Heffron,[1,2] Connie Celum,[1,2,4] Allan Ronald,[5,6] Nelly Mugo,[1,7] and Jared M. Baeten[1,2,4]; for the Partners PrEP Study Team[a]

[1]Department of Global Health, [2]Department of Epidemiology, [3]Department of Laboratory Medicine, and [4]Department of Medicine, University of Washington, Seattle; [5]Department of Medicine, and [6]Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada; and [7]Center for Clinical Research, Kenya Medical Research Institute, Nairobi

Background. Intermittent shedding of human immunodeficiency virus type 1 (HIV) in semen occurs despite effective antiretroviral therapy (ART) and suppressed blood HIV-1 RNA levels.

Methods. We assessed the frequency, magnitude, and correlates of seminal HIV-1 RNA shedding in HIV-1–infected African men initiating ART.

Results. Seminal HIV-1 RNA was detected in 24% (37 of 155), 10% (5 of 49), and 11% (8 of 70) of samples collected 0–3, 4–6, and >6 months after ART initiation. When blood HIV-1 levels were suppressed, seminal HIV-1 RNA was detected in 8% (16 of 195), and 82% (13 of 16) had an HIV-1 RNA load of < 1000 copies/mL.

Conclusions. Seminal HIV-1 RNA shedding was infrequent and present at low levels in HIV-1–infected African men with suppressed blood HIV-1 RNA.

Keywords. antiretroviral therapy; HIV-1; RNA; semen.

Semen is the principal vector for transmitting human immunodeficiency virus type 1 (HIV) from men to women [1]. 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 transmission of HIV-1 to uninfected partners [2]. 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 [3]. First-phase decay kinetics may be slower in semen than in blood, but second-phase decay rates are thought to be similar in both fluids [4].

Three HIV-1 shedding patterns have been described in semen: none, continuous, and intermittent [5]. In men who shed HIV-1 in semen, the predominant shedding pattern is intermittent [5]. 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) [6]. In men with low seminal viral loads, a probabilistic empirical model estimated a male-to-female HIV-1 transmission risk of 3 cases/10 000 sexual episodes [7].

HIV-1–infected men initiating ART require counseling about the risk of male-to-female HIV-1 transmission, including information about the 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 preexposure prophylaxis [8]. At enrollment, HIV-1–infected partners did not meet eligibility criteria for ART initiation according to national treatment guidelines. They were followed quarterly, and they underwent regular clinical monitoring, 6-monthly $CD4^+$ T-cell 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 3 months.

All men were provided with HIV-1 prevention services, including risk-reduction counseling, free condoms, and screening for and treatment of STIs 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 specimen collection were scheduled at regular intervals following study enrollment and not according to the time of ART initiation. HIV-1–infected men provided blood specimens for HIV-1 RNA quantification at enrollment, every 6 months thereafter, and at the last study visit. $CD4^+$ T-cell counts were quantified every 6 months, using flow cytometry

Received 26 January 2016; accepted 29 March 2016; published online 6 April 2016.

[a]Study team members are listed at the end of the text.

Correspondence: A. Mujugira, International Clinical Research Center, University of Washington, Box 359927, 325 Ninth Ave, Seattle, WA 98104 (mujugira@uw.edu).

The Journal of Infectious Diseases® 2016;214:212–5

DOI: 10.1093/infdis/jiw131

(BD Biosciences). Semen specimen collection was scheduled at the 6- and 12-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 600–800 *g*, 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 compliant with the Clinical Laboratory Improvement Amendments and certified by the College of American Pathologists. 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 Diagnostic) assays, and for *Trichomonas vaginalis* infection, using APTIMA TV TMA (Gen-Probe) or In Pouch TV (Biomed Diagnostics). *Treponema pallidum* serology was performed using rapid plasma reagin tests, and positive results were confirmed using *T. pallidum* particle agglutination tests. All study site laboratories participated in external quality assurance programs for the respective test.

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 or, if paired samples were not available, in blood samples collected within 3 months of collection of the semen sample. The presence of HIV-1 RNA in semen was quantified in 3 periods: 0–3, 4–6, and >6 months after ART initiation. The correlation between blood and seminal HIV-1 RNA quantity was assessed using the Spearman 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, STIs, pre-ART $CD4^+$ T-cell count, blood HIV-1 RNA concentrations, and duration of ART. Factors with P values of $\leq .20$ in bivariate analyses were included in the multivariate model. All analyses were performed using Statistical Analysis Software, 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 a semen specimen before ART, and 31 (4%) did not give a semen sample. The remaining 233 (31%) provided 280 semen samples after ART initiation. Analyses of 6 samples failed, and these samples 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 years), and the median time from ART initiation to semen HIV-1 RNA quantification was 2.83 months (IQR, 0.16–6.45 months). Most (98%) were receiving ART regimens containing a nonnucleoside reverse transcriptase inhibitor.

Characteristics of HIV-1–infected men are shown in Table 1. The median age at enrollment was 40 years (IQR, 34–47 years), and nearly all (99%) were married; the median $CD4^+$ T-cell count and HIV-1 RNA concentration were 336 cells/μL (IQR, 293–403 cells/μL) and 4.54 $\log_{10}$ copies/mL (IQR, 3.81–5.03 $\log_{10}$ copies/mL), respectively. The prevalence of STIs 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 pretreatment $CD4^+$ T-cell counts (244 vs 272 cells/μL), 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% of semen samples (50 of 274). Seminal HIV-1 RNA was detected in 24% of samples (37 of 155) collected 0–3 months after ART initiation, 10% (5 of 49) collected 4–6 months after ART initiation, and 11% (8 of

Table 1. Characteristics of 231 Human Immunodeficiency Virus Type 1 (HIV-1)–Infected Men

Characteristic	Value
Age, y	40 (34–47)
Married to study partner	230 (99)
Education duration >7 y	134 (58)
Any monthly income	185 (80)
Any unprotected sex[a]	20 (9)
$CD4^+$ T-cell count, cells/μL[a]	244 (212–313)
Blood HIV-1 RNA level, $\log_{10}$ copies/mL[a]	4.54 (3.81–5.03)
Sexually transmitted pathogen[a]	
Trichomonas vaginalis	0 (0)
Neisseria gonorrhoeae	2 (<1)
Chlamydia trachomatis	0 (0)
Treponema pallidum	2 (<1)
Duration of ART, mo	19.42 (14.0–24.7)

Data are median value (interquartile range) or no. (%) of subjects.

Abbreviation: ART, antiretroviral therapy.

[a] Time-varying covariates were assessed at the study visit prior to ART initiation.

70) collected >6 months after ART initiation. When detected, median HIV-1 RNA levels were 2.96 $\log_{10}$ copies/mL (IQR, 2.51–3.50 $\log_{10}$ copies/mL).

Semen HIV-1 RNA was detected in 8% of samples (16 of 195) obtained when blood HIV-1 RNA concentrations were suppressed, of which 11% (10 of 90), 5% (2 of 41), and 6% (4 of 64) were 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 $\log_{10}$ copies/mL). Most (82% [13 of 16]) had a semen HIV-1 RNA quantity of <1000 copies/mL. No STIs were detected at the time HIV-1 was detected in semen.

Factors Associated With Seminal HIV-1 RNA Detection

HIV-1 RNA concentrations in paired blood and semen specimens were strongly correlated (Spearman rank correlation coefficient, $\rho = 0.63$; $P < .001$). In bivariate analyses, semen HIV-1 RNA shedding was significantly associated with the concentration of HIV-1 RNA in blood ($P < .001$), pretreatment $CD4^+$ T-cell count ($P = .04$), any monthly income ($P = .009$), and duration of ART ($P = .05$; Table 2). After adjustment for potential confounding factors, the HIV-1 RNA concentration in blood significantly predicted HIV-1 RNA semen shedding (adjusted odds ratio, 2.72 per $\log_{10}$; $P < .001$).

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 shedding in semen 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 of <1000 copies per 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 values in prospective studies of men undergoing suppressive ART and receiving medically assisted reproduction services to initiate a pregnancy with HIV-1–uninfected female partners [9–11]. Among men with undetectable HIV-1 in blood, HIV-1 RNA was detected in the semen of 3.7%–19.3% of European HIV-1–infected men in serodiscordant partnerships [9–13]. 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 of <1000 copies/mL. This concentration of HIV-1 in semen is associated with a very low risk of male-to-female HIV-1 transmission [1].

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 [14]. 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 [15]. These data support the HIV-1 prevention effectiveness of suppressive ART, regardless of low-level viremia 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 ART-recipient men who have sex with men, in which 6%–8% had detectable HIV-1 RNA in semen despite suppressed HIV-1 levels in blood and absence of detectable STIs [6]. In those studies, cytomegalovirus coinfection 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, and T-cell immune activation and may reflect HIV-1 compartmentalization or incomplete ART penetration of the male genital tract [6]. Recent work indicates that intermittent shedding of HIV-1 in semen can occur within a 1-hour interval [13]. This variability may be similar to isolated episodes of low-level viremia, in which detectable blood HIV-1 RNA levels (typically, levels of < 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 shedding in semen among heterosexual African men receiving 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

Table 2. Associations With Seminal Human Immunodeficiency Virus Type 1 (HIV-1) RNA Detection

Variable	OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Age (per y)	1.03 (.99–1.07)	.13	1.05 (.99–1.10)	.08
Monthly income (any)	2.57 (1.27–5.20)	.009	1.96 (.74–5.17)	.17
Duration of ART (per mo)	0.91 (.83–1.00)	.05	0.95 (.86–1.04)	.28
$CD4^+$ T-cell count before ART (per 100 cells/μL)	1.78 (1.02–3.12)	.04	1.92 (.97–3.82)	.06
Blood HIV-1 RNA level (per $\log_{10}$ copies/mL)	2.56 (1.79–3.66)	<.001	2.72 (1.79–4.14)	<.001
N. gonorrhoeae infection	1.35 (.66–2.74)	.41	. . .	
T. vaginalis infection	1.38 (.67–2.84)	.39	. . .	

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; *N. gonorrhoeae*, *Neisseria gonorrhoeae*; OR, odds ratio; *T. vaginalis*, *Trichomonas vaginalis*.

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 among males receiving 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 who were receiving suppressive ART and had 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.

STUDY GROUP MEMBERS

Partners PrEP study team. University of Washington Coordinating Center and Central Laboratories (Seattle, WA): Connie Celum (principal investigator and protocol cochair), Jared M. Baeten (medical director and protocol cochair), Deborah S. Donnell (protocol statistician), and Robert W. Coombs, Lisa Frenkel, Craig W. Hendrix, Jairam R. Lingappa, and M. Juliana McElrath.

Study sites and site principal investigators. Eldoret, Kenya (Moi University, Indiana University): Kenneth H. Fife and Edwin Were; Kabwohe, Uganda (Kabwohe Clinical Research Center): Elioda Tumwesigye; Jinja, Uganda (Makerere University, University of Washington): Patrick Ndase and Elly Katabira; Kampala, Uganda (Makerere University): Elly Katabira and Allan Ronald; Kisumu, Kenya (Kenya Medical Research Institute, University of California–San Francisco): Elizabeth Bukusi and Craig R. Cohen; Mbale, Uganda (The AIDS Support Organization, CDC-Uganda): Jonathan Wangisi, James D. Campbell, and Jordan W. Tappero; Nairobi, Kenya (University of Nairobi, University of Washington): James Kiarie, Carey Farquhar, and Grace John-Stewart; Thika, Kenya (University of Nairobi, University of Washington): Nelly R. Mugo; and Tororo, Uganda (CDC-Uganda, The AIDS Support Organization): James D. Campbell, Jordan W. Tappero, and Jonathan Wangisi.

Data management was provided by DF/Net Research (Seattle, WA), and site laboratory oversight was provided by Contract Laboratory Services of the Wits Health Consortium (University of the Witwatersrand, Johannesburg, South Africa). Study medication was donated by Gilead Sciences.

Notes

Acknowledgments. We thank the study participants, for their participation and dedication, and the study team members at the research sites and at the University of Washington, for their contributions to data collection.

A. M. and J. M. B. designed the study and wrote the first draft. R. W. C. oversaw all HIV-1 RNA testing. A. M. 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.

Disclaimer. The funder had no role in study design; data collection, analysis, and interpretation; or writing of the report.

Financial support. This work was supported by the Bill and Melinda Gates Foundation (OPP47674), the National Institutes of Health (R01 MH095507), and the University of Washington Centers for AIDS Research (P30-AI-27757).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Baeten JM, Kahle E, Lingappa JR, et al. Genital HIV-1 RNA predicts risk of heterosexual HIV-1 transmission. Sci Transl Med **2011**; 3:77ra29.
2. Cohen MS, Smith MK, Muessig KE, Hallett TB, Powers KA, Kashuba AD. Antiretroviral treatment of HIV-1 prevents transmission of HIV-1: where do we go from here? Lancet **2013**; 382:1515–24.
3. Perelson AS, Essunger P, Cao Y, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature **1997**; 387:188–91.
4. Graham SM, Holte SE, Dragavon JA, et al. HIV-1 RNA may decline more slowly in semen than in blood following initiation of efavirenz-based antiretroviral therapy. PLoS One **2012**; 7:e43086.
5. Gupta P, Leroux C, Patterson BK, et al. Human immunodeficiency virus type 1 shedding pattern in semen correlates with the compartmentalization of viral Quasi species between blood and semen. J Infect Dis **2000**; 182:79–87.
6. Gianella S, Haubrich R, Morris SR. Suppressed but still infectious. Future Virol **2013**; 8:1037–40.
7. Chakraborty H, Sen PK, Helms RW, et al. Viral burden in genital secretions determines male-to-female sexual transmission of HIV-1: a probabilistic empiric model. AIDS **2001**; 15:621–7.
8. Baeten JM, Donnell D, Mugo NR, et al. Single-agent tenofovir versus combination emtricitabine plus tenofovir for pre-exposure prophylaxis for HIV-1 acquisition: an update of data from a randomised, double-blind, phase 3 trial. Lancet Infect Dis **2014**; 14:1055–64.
9. Bujan L, Daudin M, Matsuda T, et al. Factors of intermittent HIV-1 excretion in semen and efficiency of sperm processing in obtaining spermatozoa without HIV-1 genomes. AIDS **2004**; 18:757–66.
10. Marcelin AG, Tubiana R, Lambert-Niclot S, et al. Detection of HIV-1 RNA in seminal plasma samples from treated patients with undetectable HIV-1 RNA in blood plasma. AIDS **2008**; 22:1677–9.
11. Halfon P, Giorgetti C, Khiri H, et al. Semen may harbor HIV despite effective HAART: another piece in the puzzle. PLoS One **2010**; 5:e10569.
12. Dulioust E, Leruez-Ville M, Guibert J, et al. No detection of HIV 1-RNA in semen of men on efficient HAART in the past 4 years of a 2002–2009 survey. AIDS **2010**; 24:1595–8.
13. Ferraretto X, Estellat C, Damond F, et al. Timing of intermittent seminal HIV-1 RNA shedding in patients with undetectable plasma viral load under combination antiretroviral therapy. PLoS One **2014**; 9:e88922.
14. Sturmer M, Doerr HW, Berger A, Gute P. Is transmission of HIV-1 in non-viraemic serodiscordant couples possible? Antivir Ther **2008**; 13:729–32.
15. Mujugira A, Celum C, Coombs RW, et al. HIV-1 Transmission risk persists during the first 6 months of antiretroviral therapy. J Acquir Immune Defic Syndr **2016**; doi:10.1097/QAI.0000000000001019.