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Permissive and Protective Factors Associated With Presence, Level and Longitudinal Pattern of Cervicovaginal HIV Shedding

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Abstract

Background—Cervicovaginal HIV level (CV-VL) influences HIV transmission. Plasma viral load (PVL) correlates with CV-VL but discordance is frequent. We evaluated how PVL, behavioral, immunologic and local factors/conditions individually and collectively correlate with CV-VL.

Methods—CV-VL was measured in cervicovaginal lavage fluid (CVL) over 976 person-visits for 481 HIV-infected women in a longitudinal cohort study. We correlated identified factors with CV-VL at individual person-visits and detectable/undetectable PVL strata by univariate and multivariate linear regression, and with shedding pattern (never, intermittent, persistent 3 shedding-visits) in 136 women with 3 visits by ordinal logistic regression.

Results—450/959 (46.9%) of person-visits with available PVL were discordant. 435/959 (45.3%) had detectable PVL with undetectable CV-VL and 15/959 (1.6%) undetectable PVL with detectable CV-VL. Lower CV-VL correlated with HAART usage ($P=0.01$). Higher CV-VL correlated with higher PVL ($P<0.001$), inflammation-associated cellular changes ($P=0.03$), cervical ectopy ($P=0.009$), exudate ($P=0.005$), and trichomoniasis ($P=0.03$). In multivariate analysis of the PVL-detectable stratum, increased CV-VL correlated with the same factors and friability ($P=0.05$), while with undetectable PVL, decreased CV-VL correlated with HAART use ($P=0.04$). In longitudinal analysis, never (40.4%) and intermittent (44.9%) shedding were most frequent. Higher-frequency shedders were more likely to have higher initial PVL (OR=2.47/log₁₀ increase), HSV-2 seropositivity (OR=3.21) and alcohol use (OR=2.20).

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Author Disclosure Statement

No competing financial interests exist.

Conclusions—While PVL correlates strongly with CV-VL, discordance is frequent. When PVL is detectable, cervicovaginal inflammatory conditions correlate with increased shedding. However, genital shedding is sporadic and not reliably predicted by associated factors. HAART, by reducing PVL, is the most reliable means of reducing cervicovaginal shedding.

Introduction

Worldwide, 33.4 million people are infected with human immunodeficiency virus type 1 (HIV) with an estimated 2.7 million new infections yearly.¹ Most infections are acquired sexually or perinatally. Higher plasma viral load (PVL) correlates with increased likelihood of sexual and perinatal transmission, while antiretroviral treatment (ART), in particular highly active antiretroviral therapy (HAART), correlates with reduced transmission.²⁻⁵ HIV presence in the female genital tract is important in perinatal⁴ and sexual transmission.⁶ Increased cervicovaginal HIV level (CV-VL) likely increases risk of trans mucosal HIV passage during penile-vaginal intercourse and vaginal delivery.

The quantity of HIV in the female genital tract correlates with various factors, including PVL,⁷ immune status,⁸ cervicovaginal inflammation,⁹ and ART.^{10;11} HIV characteristics, including tropism and resistance pattern, can differ markedly in different compartments, including the female genital tract,^{12;13} male genital tract,¹⁴ and central nervous system.^{15;16} HIV can be detected in cell-free and cell-associated components of cervicovaginal fluid.¹⁷ Local conditions in the female genital tract including inflammation,¹⁸ bacterial vaginosis,¹⁹ cervical ectopy,²⁰ genital ulceration,¹⁸ candidiasis,²¹ HSV,²² trichomoniasis,²⁰ and other infections influence cervicovaginal HIV shedding. Menstrual cycle phase^{23;24} and hormonal contraception²⁵ may affect shedding. ART, in particular HAART, is associated with decreased cervicovaginal HIV shedding.^{11;26}

Discordance between HIV presence in the bloodstream and cervicovaginal shedding is frequent. HIV can be present in the genital tract despite undetectable PVL, suggesting local replication.^{27;28} Factors that may cause increased cervicovaginal shedding due to local replication include poor penetration of antiretrovirals into genital secretions,²⁹ local resistance,¹³ and local inflammation.²⁸ Conversely, HIV is sometimes absent from genital secretions despite high PVL, suggesting that additional factors may be necessary to permit shedding.

While previous studies have shown association between numerous conditions and cervicovaginal HIV shedding, the mechanisms for presence and level of HIV in the cervicovaginal compartment are not well understood.²⁸ Several studies have evaluated the variation in cervicovaginal HIV shedding over various time-periods, particularly the possible influence of the menstrual cycle.^{23;24;30;31} However, the frequencies and determinants of different temporal patterns of cervicovaginal HIV shedding have not been fully characterized. The purpose of this study is to evaluate how behavioral, therapeutic, clinical, immunologic and local factors correlate, individually and collectively, with cervicovaginal HIV shedding, at single time-points and as a longitudinal pattern.

Methods

Study Population

This sub-study was nested within the Women's Interagency HIV Study (WIHS), an ongoing multicenter, prospective study of the natural and treated history of HIV infection in women.³² WIHS participants provide informed consent for their participation in keeping with local, institutional and national guidelines. All women who had quantitation of HIV-1

in cervicovaginal lavage fluid (CVL) at least once as part of several WIHS substudies between 11/9/1994 and 9/12/2001 were included in this study.^{7;33}

Clinical Data

WIHS methods have been described.³² At baseline and each semiannual visit, subject history, including health information, medications including antiretrovirals, sexual history, substance use and other behavioral data were obtained using a standardized interview. Physical and gynecological examinations were performed. Blood and gynecological specimens were collected for local testing and repository storage. Genital tract assessment included visual inspection, speculum, and sometimes bimanual and rectal examination. Cervical lesions (ulcers, vesicles, fissures, or warts), ectopy ("beefy" redness extending from os onto cervix), friability (erythematous tissue that bleeds easily), exudate (discharge of any type) were ascertained visually. CVL was collected by spraying 10 mL of sterile, non-bacteriostatic saline against the cervical os and endocervix and aspirating from the posterior vaginal fornix. Unfractionated CVL was stored at -70°C .

Laboratory Data

Blood analyses included complete blood count, lymphocyte subsets, and plasma HIV RNA (PVL). We determined lymphocyte subsets with standard flow cytometric techniques at local laboratories. Baseline serology for HSV-1, HSV-2 and syphilis screening were performed with Western Blot and rapid plasma reagin (RPR) test respectively in a central laboratory.³² Baseline HCV antibody testing was performed by Abbott enzyme immunoassays (version 2.0 or 3.0). HCV RNA levels were measured in a single laboratory (University of Southern California) by polymerase chain reaction (Roche Diagnostics).³⁴ Baseline hepatitis B profile was also performed in local laboratories.

We collected whole blood for PVL determination in sodium citrate cell preparation tubes. Plasma HIV quantitation was completed in four central laboratories. Initially, PVL was measured with a nucleic acid sequence-based amplification technique (Organon Teknika Corp, Durham, NC), with lower threshold of detection of 4000 copies/mL. Similar methods with greater sensitivity were used as they became available. WIHS currently uses the NucliSens (Organon Teknika Corp) assay for quantification of HIV RNA in plasma with a lower limit of detection (LLD) of 80 copies/mL with 1 mL of sample input. In general, at the beginning of the study period (visits 1–7), PVL had an LLD of 4000 copies/mL, while for visits 7–9 it improved to 400 copies/mL, and from visit 10 onward to 80 copies/mL. HIV quantitation in CVL (CV-VL) used the NucliSens assay (LLD of 80 copies/mL). However, these changes occurred in the laboratories at different times.

Cervicovaginal specimens included vaginal swabs for pH, potassium hydroxide preparation, candida culture, saline preparation for microscopy, Gram stain, swabs for HSV culture if cervical/vaginal ulcer, fissure, or vesicle present, syphilis if ulcer/fissure/vesicle(s) present, endocervical swabs for gonorrhea/chlamydia nucleic acid detection tests and trichomonas culture, and a Papanicolaou (Pap) smear. CVL was tested for microscopic blood and semen. Pap smears were read in a central laboratory (Kyto Diagnostics New York, N.Y.). Squamous metaplasia (replacement of one normal type of epithelium with another), endocervical cells, inflammation (leukocytes on Pap smear), and inflammation-associated cellular changes (cellular changes found with inflammation including basophilic cytoplasm, enlarged unevenly-sized nuclei, enlarged, irregular or multiple nucleoli, repair) were ascertained from Pap smear. Gram stains were interpreted for bacterial vaginosis (BV) in a central laboratory (University of Washington) using the Nugent score criteria, with categorization as normal (0–3), intermediate (4–6), or consistent (7–10).³⁵ Trichomoniasis was diagnosed if motile trichomonads were present on wet mount or with positive culture. Candidiasis was

diagnosed by pseudohyphae presence on potassium hydroxide preparation or positive culture. PCR to identify 29 types of human papillomavirus (HPV) was performed in central laboratories.³⁶ We analyzed separately several high-risk types of HPV (16, 18, 31, 33, 35).

Statistical Methods

Demographic covariates included age (categorized as <35, 35–40, 41+) and self-identified race/ethnicity (White, African-American, Hispanic, Other). HIV exposure category (intravenous drug use, heterosexual risk, transfusion risk, no identified risk, unknown) was specified. Behavioral covariates included current smoking (no, yes), current alcohol consumption (abstainer, <3, 3–13, 14 drinks/week), current injection drug use (no, yes), current use of other recreational substances (marijuana, cocaine, heroin, other), number of lifetime male sex partners (0–6, 7–29, 30+), number of male sex partners since last study visit (0, 1, 2+), and vaginal sex with male in past 48 hours (no, yes). Therapeutic, immunologic, and clinical covariates included type of ART used since last visit (none, monotherapy, combination therapy, HAART), hormonal contraceptive use in past 6 months (no, yes), PVL, CD4 cell count (< 200, 201–350, 351–500, and >500 cells/mm³), prior history of an AIDS-defining illness, hepatitis C status at baseline (antibody negative, antibody positive/RNA negative, antibody positive/RNA positive), and seropositivity for HSV-1 and HSV-2. The definition of HAART was guided by the DHHS/Kaiser Panel 2008.³⁷ Local cervicovaginal covariates included vaginal pH (<4.5, 4.5–5.4, 5.5), abnormal Pap (no, yes), bacterial vaginosis score,³⁵ and presence of the following: friability, ectopy, exudate, lesions, candidiasis, *Trichomonas vaginalis*, endocervical cells, squamous metaplasia, cervicovaginal inflammation, inflammation-associated cellular changes, and HPV (all types, oncogenic types).

Individual visit analysis, level of shedding—This analytic approach correlated data from individual person-visits with level of CV-VL. To accommodate multiple visits per subject, regression models utilized generalized estimating equations with an exchangeable correlation matrix and an identity link function. The dependent variable for this linear regression model was log₁₀CV-VL. Visits where the CV-VL was less than the LLD (80 copies/ml) were assigned a value of ½ LLD (40). Visit-specific covariates were used from each visit at which CV-VL was measured. Plasma HIV RNA was analyzed both as categorical (< 4000, 4001–9999, 10000–39999, 40000–99999, 100,000 copies/mL), where 4000 copies/mL was modeled as the referent group, and as a log₁₀-transformed continuous variable, where values less than the LLD for the assay used were assigned a numeric value of ½LLD. Factors were evaluated in unadjusted and multivariate models. The multivariate model retained factors associated with CV-VL in unadjusted analyses at *P*<0.10 that remained at *P*<0.10. Associations were summarized as β-coefficients with associated standard errors. Two-sided hypotheses were assessed at the 5% significance level. Finally, we stratified person-visits into groups with detectable (> 80 copies/ml) and undetectable (<80 copies/ml) PVL and performed similar analyses on these strata to determine shedding associations with PVL detectable or undetectable. Person-visits with undetectable PVL when LLD was 400 copies/mL (n=24) and 4000 copies/mL (n=10) were excluded from this analysis.

In order to better understand the role of cervicovaginal inflammation as a condition in shedding we devised a tool to assess the presence/absence of any cervicovaginal inflammatory condition associated with shedding. We defined a binary variable, called inflammation summary variable (ISV), to have the value “1” if any local inflammatory factor significantly associated with CV-VL on univariate analysis was present (inflammation-associated cellular changes, cervical ectopy, exudate, friability, lesions, vaginal pH >5.5, intermediate BV score, consistent BV score, trichomoniasis) and “0” if

none was present. Using this variable, we used Fisher's exact test to evaluate for interaction between PVL detectability and inflammation (by ISV value) in correlation with shedding presence (Table 2). We used the generalized estimating equation to test for interaction between cervicovaginal inflammation and PVL in correlation with CV-VL.

Analysis of longitudinal shedding pattern—This analytic approach categorized subjects into three groups to evaluate the pattern of cervicovaginal shedding over a series of visits. Categories were defined as follows: “never shedder” if a subject had no visits at which HIV was detected in cervicovaginal secretions, “intermittent shedder” if she had shedding at one or two visits, “persistent shedder” if she had shedding at three or more visits. Shedding category was assigned a three-level variable (coded 0, 1, 2) corresponding to never, intermittent and persistent shedding, respectively. For uniformity, this analysis was restricted to all subjects with at least three visits over a three-year period where at most one semiannual visit could be missed between evaluable visits. Data included visits from 3/31/1998–9/12/2001. Independent variables were the same as in the individual visit analysis, but taken from the first evaluable visit. PVL was analyzed as a categorical variable (< 400, 401–3999, 4000–19999, and ≥ 20000 copies/mL) and as a log₁₀-transformed continuous variable, where values less than the LLD for a particular assay were assigned a numeric value of ½ LLD. Summary variables were created to characterize the pattern of HAART use (none, intermittent, always) and PVL detection (always, sometimes, never) over the evaluated visits. Initial unadjusted analyses were performed using ordinal logistic regression followed by multivariate analyses, using all factors with *P*<0.10 in univariate analysis, controlling for type of ART (none, non-HAART, HAART) and PVL category. Similarly, unadjusted analyses and analyses adjusting for initial PVL were performed on the subgroup of patients with “always-detectable PVL,” but not on the “sometimes” and “never-detectable” groups, due to insufficient cases of persistent shedding (2/50 and 0/24, respectively).

Results

Study Population

481 women had a total of 976 visits at which genital shedding was evaluated. Baseline demographic and clinical characteristics of the study population are summarized in Table 1. The median number of person-visits was 1. 31% had 3 or more person-visits. The median age at baseline was 36.3 years. 52% of women were African American and 33% Hispanic. 29.6% had baseline plasma viral load below 4000 copies/mL (median=16,000 copies/mL), while 25.5% had CD4 < 500 cells/mm³. Tobacco use was reported at 38% of person-visits. Hormonal contraception was reported at 6% of person-visits. The distribution of risky behaviors included: heavy alcohol use, 11%, injection drug use, 36.5%, and >30 lifetime sex partners, 35.9%. Few women had evidence of chlamydia, 0.2%, gonorrhea, 0%, or active HSV at baseline, 0.2%. HSV seropositivity was common, HSV-1: 83%; HSV-2: 77%. 17.2% had a positive screening test for syphilis (RPR). Other cervicovaginal infections present at baseline included trichomoniasis, 11.8%, HPV (all types) 52.7%, bacterial vaginosis (Nugent score 7–10) 47.1%. The percentage of participants receiving HAART increased from 0.3% at the initial visit where shedding was measured to 70% by the last visit of the study period.

Discordance between PVL and CV-VL

Subgroups of person-visits stratified with respect to PVL, CV-VL, cervicovaginal inflammation and antiretroviral therapy are shown in Table 2. Discordance between PVL and CV-VL occurred in 47% of person-visits. In 959 person-visits with measured PVL, 45.3% had detectable PVL/undetectable CV-VL, 32.6% detectable PVL/detectable CV-VL,

20.4% undetectable PVL and CV-VL, and 1.6% had undetectable PVL/detectable CVL. Significant inflammation, expressed by the “inflammation summary variable,” was present in 76.5% and absent at 23.5% of evaluable person-visits (n=958). CV-VL was detectable in 37.4% of person-visits at which inflammation was present (ISV=1) and 24% at which it was absent (ISV=0). When PVL was detectable, CV-VL was detectable in 264/587 (44.9%) of person-visits with inflammation presence and 48/160 (30%) with absence. In the sub-group with PVL 80 (LLD=80), shedding was significantly more likely with inflammation present (ISV=1) ($P=0.003$).

Factors associated with level of shedding at individual person-visits

Table 3 shows variables associated with CV-VL level at individual person-visits. In multivariate analysis, higher CV-VL correlated significantly with higher PVL ($\beta=0.50$ per \log_{10} copies/mL; $P<0.001$), cervical inflammation-associated cellular changes ($\beta=0.38$; $P=0.03$), ectopy ($\beta=0.48$; $P=0.009$), exudate ($\beta=0.18$; $P=0.005$), and trichomoniasis ($\beta=0.31$; $P=0.03$). Lower CV-VL correlated with HAART use ($\beta=-0.17$; $P=0.01$). CV-VL did not correlate with hormonal contraception use. The inflammatory summary variable correlated with increased CV-VL in univariate analysis ($\beta=0.29$; $P<0.001$), and in a separate multivariate model with \log_{10} HIV-RNA and antiretroviral therapy ($\beta=0.16$; $P=0.004$). PVL had significant interaction with the inflammation summary variable in correlating with CV-VL (interaction coefficient 0.26, $P=0.006$). Multivariate analysis (Table 4) of the PVL-detectable stratum (n=665) showed strong correlation between higher CV-VL and PVL (for $>100,000$ copies/mL $\beta=0.80$; $P<0.001$), friability ($\beta=0.23$; $P=0.05$), ectopy ($\beta=0.46$; $P=0.02$), exudate ($\beta=0.25$; $P=0.001$), trichomoniasis ($\beta=0.33$; $P=0.04$), and inflammation-associated cellular changes ($\beta=0.61$; $P=0.007$). In multivariate analysis of the PVL-undetectable stratum (n=132 person-visits), cervicovaginal inflammatory conditions did not correlate with CV-VL. As expected, HAART correlated with decreased CV-VL ($\beta=-0.39$; $P=0.04$).

Factors associated with pattern of genital shedding over multiple visits (Table 5)

Of 481 evaluable women, 136 (31%) had CVL-VL measured at 3 or more visits within a 3-year period with a median of four evaluable visits (range: 3–6). The shedding distribution included: 40.4% (n=55) “never,” 44.9% (n=61) “intermittent,” and 14.7% (n=20) “persistent.” Ordinal logistic regression adjusted for ART showed that a pattern of higher frequency of shedding over visits (i.e., intermittent/persistent vs. never; or persistent vs. intermittent/never) was associated with higher initial PVL (OR= 2.47 per \log_{10} copies/mL; $P<0.01$; Table 5). With adjustment for ART and PVL, higher shedding frequency also correlated with any alcohol use (OR=2.20; $P=0.03$) and seropositivity for HSV-2 (OR=3.21; $P=0.009$). Never detectable PVL correlated strongly with lower likelihood of higher shedding frequency (OR=0.10; $P<0.001$). Even in the subgroup with always detectable PVL, 18/62 (29%) had persistent shedding, but 20/62 (32%) never shed and 24/62 (39%) shed intermittently. In this subgroup, higher shedding frequency correlated with alcohol use (OR=4.92; $P=0.003$) and HSV-2 seropositivity (OR=4.44; $P=0.04$) (Table 6, supplemental digital content). Additionally in this subgroup, vaginal candidiasis correlated with a 15-fold increase in the odds of higher shedding frequency (OR=15.14; $P=0.009$).

Discussion

This study comprehensively assessed demographic, behavioral, clinical, therapeutic and local factors that correlate individually and collectively with level and pattern of HIV shedding in the female genital tract both at individual person-visits (cross-sectionally) and longitudinally. The two analytic approaches are complementary. The person-visit analysis elucidates the association between identified factors and CV-VL at individual time-points, while the longitudinal analysis clarifies how behaviors and conditions present at the initial

visit, as well as summary variables measuring HAART adherence and PVL suppression over all visits, correlate with the temporal pattern of shedding. Subanalyses shed light on discordance between PVL and cervicovaginal HIV presence, level, and pattern.

As shown previously,^{11;26;38} at individual person-visits PVL and HAART are the principal factors associated with CV-VL, correlating with higher and lower levels, respectively. Correlation between PVL and CV-VL may be direct, due to transmigration of cell-free or cell-associated HIV from the bloodstream, indirect, related to local replication responding to the same factors as systemic replication, or both. Similarly, HAART may influence CV-VL directly by reducing local replication, indirectly by reducing HIV bloodstream replication, or both. Consistent with previous studies,^{18;39;40} local inflammatory conditions, diagnosed clinically, such as exudate, ectopy, friability, and presence of lesions, microbiologically, such as *Trichomonas vaginalis*, and histologically, such as inflammation-related cellular changes correlated significantly with increased shedding in the person-visit analysis.

How local inflammatory conditions lead to increased cervicovaginal HIV shedding is not well understood. There may be several mechanisms, and their order of importance may vary depending on circumstances. Cervicovaginal inflammation may increase vascular permeability, allowing HIV transmigration from bloodstream to cervicovaginal compartment.¹⁸ Local inflammation may directly stimulate HIV replication, or lead to recruitment of HIV-producing leukocytes from adjacent lymphoid tissue.^{22;39} When HIV is undetectable in the bloodstream, local replication, allowed by inadequate antiretroviral levels or resistance, may lead to detectable HIV in cervicovaginal secretions. Some authorities suggest that the source of most inflammation-associated cervicovaginal HIV is local replication.^{18;39}

There was significant discordance between PVL and CV-VL both at individual person-visits and as a pattern. Similar to previous studies,^{28;41} CV-VL was detectable at 7.6% (15/211) of person-visits when PVL was undetectable. Surprisingly, CV-VL was undetectable at 58% (436/748) of person-visits when PVL was detectable. With detectable PVL, CV-VL correlated significantly with inflammatory conditions and PVL. Indeed, inflammation presence (ISV=1) led to increased correlation between PVL and CV-VL. This suggests that, when local inflammatory conditions are present, a significant amount of HIV in cervicovaginal secretions is due to transmigration from the bloodstream, rather than local replication, likely due to a compromised bloodstream-tissue barrier. However, even in the absence of cervicovaginal inflammation, CV-VL was sometimes detectable. Conversely, in the undetectable PVL stratum, increased shedding did not correlate with local inflammatory conditions. This suggests that, when antiretroviral suppression is effective, cervicovaginal inflammatory conditions are insufficient to cause shedding. HAART correlated with decreased CV-VL in the entire group and was the only factor to correlate with decreased CV-VL in the PVL-undetectable stratum. Though the main protective effect of HAART stems from PVL suppression, an additional protective effect may be suppression of cervicovaginal HIV replication, particularly when PVL is already low or undetectable. However, the number of person-visits with undetectable PVL was insufficient to draw reliable conclusions about all but very strong associations in this stratum.

In longitudinal analysis, even with always undetectable PVL, intermittent CVL shedding was sometimes present (5/24 subjects). Conversely, many subjects with always detectable PVL never had CVL HIV shedding (20/62 subjects). Persistent shedding in this group was associated with inflammatory factors such as HSV-2 seropositivity and vaginal candidiasis, as well as alcohol use. Thus, the longitudinal pattern of shedding correlates not only with HIV levels in the bloodstream, but also with factors leading to cervicovaginal inflammation.

Interestingly, we noted an association between alcohol use and shedding persistence. Association between alcohol use and cervicovaginal shedding was demonstrated in one previous study.⁴² This is plausible, as alcohol affects HIV replication and susceptibility, causing increased SIV replication in animal models,⁴³ and increased HIV replication in PBMCs and susceptibility of CD4 lymphocytes to HIV infection *in vitro*.⁴⁴ No other behavior, including recent sexual intercourse, was associated with increased cervicovaginal shedding. Similar to previous studies,⁴⁵ HSV-2 seropositivity without overt lesions correlated with shedding persistence. This may be due to inflammation from low-grade HSV replication.

Our study has several limitations. An observational study, it cannot determine causation. During the study period there were major changes in HIV quantitation and treatment and resultant population health status. HIV levels in cervicovaginal secretions were measured in CVL, a semiquantitative method, rather than more precise means, such as cervical wick or swab. In addition, presence of intracellular integrated HIV-provirus was not evaluated, possibly leading to underestimation of HIV quantity in CVL.

Conclusions

Undetectable PVL due to effective HAART is strongly associated with reduced CV-VL, but does not assure shedding absence.²⁸ Conversely, cervicovaginal HIV shedding may be undetectable without antiretroviral therapy and with high PVL. When HIV is present in the bloodstream, “permissive” factors, conditions or behaviors associated with cervicovaginal inflammation, correlate with increased shedding. “Protective” factors include HAART and control of such conditions or behaviors. Therefore, prediction of cervicovaginal HIV shedding solely on the basis of ART and PVL is unreliable. As a practical matter, HIV-infected women should be counseled that cervicovaginal inflammatory conditions may increase risk of sexual transmission of HIV, and medical providers advised to diagnose and treat such conditions as a means of reducing HIV transmission. Serodiscordant couples with perfect HAART adherence and consistently undetectable PVL in the infected partner should be advised that while sexual transmission is unlikely, consistent condom use combined with HAART remains the most reliable means of prevention.^{2;46} Further studies are needed to determine the source of HIV in cervicovaginal secretions, and factors that lead to shedding despite control of systemic replication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The Woman's Interagency HIV Study Protocol was reviewed and approved by the institutional review boards at each participating center, and written and informed consent was obtained from all patients. Human experimentation guidelines of the U.S. Department of Health and Human Services were followed in the conduct of this research.

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Table 1

Demographic and Clinical Characteristics of Study Population at Baseline Visit (N=481).

Characteristic	No. of participants	No. (%) ^a	Median (IQR)
Age, years	481		36.3 (31.7–41.5)
<35		204 (42.4)	
35–40		150 (31.2)	
41+		127 (26.4)	
Self-identified ethnicity	481		
White (Non-Hispanic)		60 (12.5)	
African-American (Non-Hispanic)		252 (52.4)	
Hispanic		159 (33.1)	
Other		10 (2.1)	
HIV exposure category	477		
Intravenous drug use		174 (36.5)	
Heterosexual contact		212 (44.4)	
Transfusion		6 (1.3)	
Not identified		85 (17.8)	
Antiretroviral therapy	480		
None		178 (37.1)	
Monotherapy		178 (37.1)	
Combination therapy		122 (25.4)	
HAART		2 (0.4)	
Plasma HIV-1 RNA level, copies/mL	473		16,000 (4,000–68,000)
4,000		140 (29.6)	
4,001–9,999		56 (11.8)	
10,000–39,999		97 (20.5)	
40,000–99,999		98 (20.7)	
100,000		82 (17.3)	
CD4 cell count, cells/mm ³	466		339 (203–512)
200		115 (24.7)	
201–350		133 (28.5)	
351–500		99 (21.2)	
>500		119 (25.5)	
Alcohol consumption	471		
None		201 (42.7)	
Light (<3 drinks/week)		146 (31.0)	
Moderate (3–13 drinks/week)		72 (15.3)	
Heavy (14 or more drinks/week)		52 (11.0)	
Injected drugs in past 6 mo	480	63 (13.1)	
Number of lifetime male sex partners	479		15 (5–50)
0–6		156 (32.6)	
7–29		151 (31.5)	

Characteristic	No. of participants	No. (%) ^a	Median (IQR)
30+		172 (35.9)	
Number of male sex partners in past 6 mo	481		1 (0–1)
0		147 (30.6)	
1		262 (54.5)	
2 or more		72 (15.0)	
Bacterial vaginosis Gram stain	471		
Normal (0–3)		169 (35.9)	
Intermediate (4–6)		80 (17.0)	
Consistent (7–10)		222 (47.1)	
Candidiasis	468	44 (9.4)	
Hepatitis C	471		
AB-negative		275 (58.4)	
AB-positive RNA-negative		34 (7.2)	
AB-positive RNA-positive		162 (34.4)	
Herpes simplex virus, type 1	465	387 (83.2)	
Herpes simplex virus, type 2	464	357 (76.9)	
Human papillomavirus, all types	203	107 (52.7)	
Human papillomavirus, types 16, 18, 31, 33, 35	203	30 (14.8)	
Trichomonas vaginalis	468	55 (11.8)	
Syphilis (+ RPR)	478	82 (17.2)	

NOTE. AB, antibody; HAART, highly active antiretroviral therapy; IQR, interquartile range.

^aPercentages exclude unknown results and may not add up to 100% due to rounding.

Table 2
 Clinical Characteristics of Study Population by Plasma Viral Load Assay LLD and Cervicovaginal Inflammation (ISV) (N person-visits=959).^a

	ISV ^c			PVL		CV-VL		Antiretroviral therapy		
	PVL	CV-VL	N	0	1	Median (range)	Median (range)	None	Non-HAART	HAART
<i>PVL LLD = 4000</i>										
<4000	<80	9	2	7	N/A	N/A	N/A	5 (55%)	4 (44%)	0
<4000	80	1	0	1	N/A	1,300		1 (100%)	0	0
			P-value **: 1.00							
4000	<80	90 ^b	14	76	47000 (4,200–2,100,000)	N/A		25 (28%)	64 (72%)	0
4000	80	111	17	94	59000 (4,600–4,900,000)	3100 (80–660,000)		27 (24%)	83 (75%)	1 (1%)
			P-value **: 1.00							
Total		211						58 (28%)	151 (72%)	1 (0.5%)
<i>PVL LLD = 400</i>										
<400	<80	23	7	16	N/A	N/A		3 (13%)	4 (17%)	16 (70%)
<400	80	1	1	0	N/A	5,400		1 (100%)	0	0
			P-value **: 0.33							
400	<80	45	11	34	8600 (500–290,000)	N/A		13 (29%)	11 (24%)	21 (47%)
400	80	32	3	29	24500 (590–1,900,000)	1900 (83–290,000)		15 (47%)	8 (25%)	9 (28%)
			P-value **: 0.14							
Total		101						32 (32%)	23 (23%)	46 (45%)
<i>PVL LLD = 80</i>										
<80	<80	164	50	114	N/A	N/A		20 (12%)	16 (10%)	128(78%)
<80	80	13	5	8	N/A	1100 (100–6,300)		5 (38%)	2 (15%)	6(47%)
			P-value **: 0.55							
80	<80	301 [*]	87	213	3100 (82–590,000)	N/A		115 (38%)	65 (22%)	121(40%)
80	80	169	28	141	13000 (81–4,600,000)	1100 (82–270,000)		81 (48%)	34 (20%)	54(32%)
			P-value **: 0.003							
Total		647						221 (34%)	117 (18%)	309(48%)

All visits

PVL	CV-VL	ISV ^c		PVL		CV-VL		Antiretroviral therapy	
		0	1	Median (range)	Median (range)	None	Non-HAART	HAART	
UD	<80	59	137	N/A	N/A	28 (14%)	24 (12%)	144 (74%)	
UD	80	6	9	N/A	1,300 (100–6,300)	7 (47%)	2 (13%)	6 (40%)	
D	<80	436*	323	5,650 (82–2,100,000)	N/A	153 (35%)	140 (32%)	142 (33%)	
D	80	48	264	29,000 (81–4,900,000)	14,000 (80–660,000)	123 (39%)	125 (40%)	64 (21%)	
Total		959	733			311 (32%)	291 (30%)	356 (38%)	

PVL=plasma viral load; CV-VL=cervicovaginal viral load; HAART=highly active antiretroviral therapy; LLD=lower limit of detection; UD=undetectable; D=detectable.

^a HIV viral load is unknown for 17 person-visits which are excluded from this table.

^b Antiretroviral therapy is unknown for one subject.

^c ISV=inflammation summary variable: value is 1 if any of the following is present at person-visit: inflammation-associated cellular changes, cervical ectopy, exudate, friability, lesions, vaginal pH >5.5, intermediate BV (Nugent) score, consistent BV score, or trichomoniasis, 0 if none is present.

* ISV is unknown for one visit for PVL LLD=80, PVL >=80, CV-VL <80 and for all visits PVL = D, CV-VL <80

** Fisher's exact tests are used to test associations between ISP and CV-VL for different levels of PVL (detectable, undetectable) in LLD categories.

Table 3
 Association of Demographic, Behavioral, Virologic, and Clinical Factors with HIV-1 RNA Level (\log_{10} copies/mL) in CVL Among 481 Participants Across 976 Visits.

Factor ^a	Univariate models ^b		Multivariate model excluding summary inflammation ^c		Multivariate model including summary inflammation ^d		
	Mean SE	β (SE)	P	β (SE)	P	β (SE)	P
Self-identified ethnicity							
White (non-Hispanic)	2.08 (0.09)	Ref	-	-	-	-	-
African-American (non-Hispanic)	2.23 (0.05)	0.15 (0.10)	0.15	-	-	-	-
Hispanic	2.32 (0.08)	0.24 (0.12)	0.04	-	-	-	-
Other	2.08 (0.18)	-0.002 (0.20)	0.99	-	-	-	-
Antiretroviral therapy							
No therapy	2.28 (0.06)	Ref	Ref	Ref	Ref	Ref	Ref
Monotherapy	2.42 (0.10)	0.13 (0.11)	0.23	0.03 (0.11)	0.78	0.03 (0.11)	0.79
Combination therapy	2.46 (0.09)	0.18 (0.10)	0.09	0.08 (0.10)	0.43	0.09 (0.09)	0.33
HAART	1.90 (0.04)	-0.38 (0.07)	<0.001	-0.17 (0.07)	0.01	-0.19 (0.07)	0.005
Plasma HIV-1 RNA, copies/mL (PVL)							
4,000	1.83 (0.03)	Ref	Ref	Ref	Ref	Ref	Ref
4,001–9,999	2.09 (0.08)	0.26 (0.09)	0.003	0.27 (0.09)	0.002	0.22 (0.08)	0.01
10,000–39,999	2.38 (0.08)	0.55 (0.09)	<0.001	0.47 (0.09)	<0.001	0.47 (0.09)	<0.001
40,000–99,999	2.60 (0.10)	0.77 (0.11)	<0.001	0.74 (0.11)	<0.001	0.68 (0.11)	<0.001
100,000	3.02 (0.12)	1.19 (0.12)	<0.001^d	1.09 (0.14)	<0.001^d	1.09 (0.13)	<0.001^d
Per \log_{10} increase		0.55 (0.04)	<0.001	0.50 (0.05)	<0.001	0.50 (0.05)	<0.001
CD4 cell count, cells/mm ³							
0–200	2.50 (0.09)	0.52 (0.10)	<0.001^d	-	-	-	-
201–350	2.32 (0.07)	0.34 (0.09)	<0.001	-	-	-	-
351–500	2.11 (0.06)	0.13 (0.07)	0.07	-	-	-	-
>500	1.98 (0.05)	Ref	Ref	-	-	-	-
Hepatitis C status							
AB-negative	2.26 (0.05)	Ref	Ref	-	-	-	-
AB-positive RNA-negative	2.06 (0.12)	-0.19 (0.13)	0.14	-	-	-	-

Factor ^a	Univariate models ^b			Multivariate model excluding summary inflammation ^c			Multivariate model including summary inflammation ^d		
	Mean (SE)	β (SE)	P	β (SE)	P	P	β (SE)	P	P
AB-positive RNA-positive	2.21 (0.07)	-0.05 (0.09)	0.54	-	-	-	-	-	-
Vaginal pH									
<4.5	2.13 (0.06)	Ref		-	-	-	-	-	-
4.5-5.4	2.18 (0.05)	0.05 (0.07)	0.51	-	-	-	-	-	-
5.5+	2.34 (0.06)	0.21 (0.08)	0.01^e	-	-	-	-	-	-
Squamous metaplasia	2.26 (0.05)	0.10 (0.06)	0.10	-	-	-	-	-	-
Inflammation	2.36 (0.13)	0.16 (0.13)	0.22	-	-	-	-	-	-
Inflammation-associated cellular changes	2.62 (0.17)	0.42 (0.17)	0.01	0.38 (0.17)	0.03				
Cervical friability	2.56 (0.10)	0.37 (0.11)	0.001						
Cervical ectopy	2.92 (0.21)	0.71 (0.21)	0.001	0.48 (0.18)	0.009				
Cervical exudate	2.39 (0.07)	0.20 (0.07)	0.007	0.18 (0.06)	0.005				
Cervical lesions	2.58 (0.14)	0.37 (0.15)	0.01	-	-	-	-	-	-
Herpes simplex virus, type 1	2.27 (0.04)	0.21 (0.09)	0.02	-	-	-	-	-	-
BV Gram stain score									
Normal (0-3)	2.12 (0.05)	Ref		-	-	-	-	-	-
Intermediate (4-6)	2.30 (0.07)	0.18 (0.08)	0.03	-	-	-	-	-	-
Consistent (7-10)	2.31 (0.06)	0.19 (0.08)	0.01^f	-	-	-	-	-	-
Trichomonas vaginalis	2.52 (0.14)	0.33 (0.15)	0.02	0.31 (0.14)	0.03				
Inflammation Summary Variable (ISV)	2.29 (0.04)	0.29 (0.06)	<0.001				0.16 (0.06)^h	0.004^h	

NOTE. Boldface type indicates statistical significance. ART, antiretroviral therapy; BV, bacterial vaginosis; HAART, highly active antiretroviral therapy; Ref, reference; SE, standard error.

^aAll factors were evaluated but only those with associations where $P < 0.10$ are included in the table.

^bLinear regression with generalized estimating equations assuming an exchangeable correlation matrix and identity link is used to estimate β -coefficients, standard errors and P values.

^cThe multivariate model includes all evaluated factors where $P < 0.10$ in the univariate model (excluding summary inflammation) that remained $P < 0.10$ in the multivariate model. Estimates are displayed for all variables that were included in the model. 857 observations are included in the multivariate model.

^d P -trend < 0.001;

^e P -trend < 0.01;

^f P -trend < 0.05.

^gThe multivariate model includes all factors in the table (except those inflammatory variables that are used to define the summary inflammation) where $P < 0.10$ in the univariate model that remained $P < 0.10$ in the multivariate model. Estimates are displayed for all variables that remained in the model. 957 observations are included in the multivariate model.

^hThere was significant interaction between ISV and PVL in correlation with CV-VL with correlation coefficient 0.26. Using Generalized Estimating Equation the following equation was derived: $Y = 0.7 + 0.33X_1 - 0.79X_2 + 0.26X_1 * X_2$ where $Y = \log_{10} \text{ CV-VL}$, $X_1 = \log_{10} \text{ HIV RNA}$, $X_2 = \text{ISV}$ ($P = 0.006$)

Table 4

STRATA. Association of Factors with HIV-1 RNA Level (\log_{10} copies/mL) in CVL at individual patient visits, stratified by Plasma HIV-RNA (PVL), Univariate and Multivariate analyses

Factor ^d	Plasma HIV-1 RNA < 80 copies/mL				Plasma HIV-1 RNA ≥ 80 copies/mL				PVL < 80 (N=132)		PVL ≥ 80 (N=665)		
	No.	No. (%)	β (SE)	P	No.	No. (%)	β (SE)	P	β (SE)	P	β (SE)	P	
Antiretroviral therapy	177				747								
No therapy		25 (14.1)	Ref			276 (14.1)	Ref						
Monotherapy		8 (4.5)	-0.36 (0.16)	0.02		123 (16.5)	0.19 (0.12)	0.12			-0.52 (0.20)	0.009	
Combination therapy		10 (5.7)	-0.05 (0.26)	0.85		142 (19.0)	0.20 (0.11)	0.07			-0.004 (0.37)	0.99	
HAAART		134 (75.7)	-0.32 (0.16)	0.04		206 (27.6)	-0.25 (0.09)	0.004			-0.39 (0.19)	0.04	
Plasma HIV-1 RNA level					748								
80–4000 copies/mL						239 (32.0)	Ref					Ref	
4,001–9,999						122 (16.3)	0.17 (0.09)	0.08				0.22 (0.10)	0.03
10,000–39,999						161 (21.5)	0.45 (0.09)	<0.001				0.40 (0.10)	<0.001
40,000–99,999						118 (15.8)	0.67 (0.11)	<0.001				0.71 (0.11)	<0.001
100,000						108 (14.4)	1.08 (0.13)	<0.001^d				1.02 (0.14)	<0.001^d
Per \log_{10} increase							0.50 (0.05)	<0.001				0.48 (0.05)	<0.001
CD4 cell count, cells/mm ³	174				728								
<200		8 (4.6)	-0.11 (0.04)	0.01		201 (27.6)	0.40 (0.12)	0.001					
201–350		29 (16.7)	-0.04 (0.07)	0.54		205 (28.2)	0.32 (0.11)	0.004					
351–500		39 (22.4)	-0.002 (0.07)	0.98		154 (21.2)	0.11 (0.09)	0.25					
>500		98 (56.3)	Ref			168 (23.1)	Ref						
Vaginal pH	176				736								
<4.5		65 (36.9)	Ref			192 (26.1)	Ref						
4.5–5.4		71 (40.3)	-0.004 (0.06)	0.95		279 (37.9)	0.05 (0.09)	0.6					
5.5+		40 (22.7)	0.08 (0.08)	0.32		265 (36.0)	0.18 (0.10)	0.09					
Squamous metaplasia	176	98 (55.7)	0.0008 (0.06)	0.99	734	484 (65.9)	0.10 (0.08)	0.2					
Inflammation	176	10 (5.7)	0.34 (0.21)	0.12	734	55 (7.5)	0.17 (0.16)	0.29					
Inflammation changes	176	8 (4.6)	0.01 (0.11)	0.91	734	32 (4.4)	0.61 (0.21)	0.004				0.61 (0.22)	0.007
Cervical friability	161	21 (13.0)	-0.009 (0.09)	0.91	676	103 (15.2)	0.40 (0.12)	0.001				0.23 (0.12)	0.05

Factor ^a	Plasma HIV-1 RNA < 80 copies/mL			Plasma HIV-1 RNA ≥ 80 copies/mL			PVL < 80 (N=132)			PVL ≥ 80 (N=665)		
	No.	No. (%)	β (SE)	P	No.	No. (%)	β (SE)	P	β (SE)	P	β (SE)	P
Cervical ectopy	161	1 (0.6)	-	-	675	36 (5.3)	0.60 (0.22)	0.005	-	-	0.46 (0.20)	0.02
Cervical exudate	161	53 (32.9)	0.002 (0.06)	0.98	678	222 (32.7)	0.27 (0.09)	0.002	-	-	0.25 (0.08)	0.001
Cervical lesions	158	6 (3.8)	-0.12 (0.03)	0.001	656	59 (9.0)	0.39 (0.17)	0.02	-0.16 (0.06)	0.01	-	-
Herpes simplex virus, type 1	166	115 (69.3)	-0.03 (0.07)	0.71	733	608 (83.0)	0.17 (0.11)	0.13	-	-	-	-
Herpes simplex virus, type 2	165	110 (66.7)	0.13 (0.05)	0.01	729	563 (77.2)	0.07 (0.11)	0.52	0.11 (0.06)	0.09	-	-
BV Gram stain score	159				707							
		88 (55.4)	Ref			270 (38.2)	Ref		Ref			
		38 (23.9)	-0.11 (0.07)	0.11		139 (19.7)	0.26 (0.10)	0.01	-0.11 (0.08)	0.13		
		33 (20.8)	-0.14 (0.06)	0.02		298 (42.2)	0.18 (0.09)	0.05	-0.19 (0.08)	0.02		
Trichomonas vaginalis	176	6 (3.4)	0.16 (0.24)	0.49	734	66 (9.0)	0.30 (0.16)	0.06	-	-	0.33 (0.16)	0.04
Summary inflammation	177	122 (68.9)	-0.09 (0.08)	0.23	747	587 (78.6)	0.37 (0.07)	<0.001				

NOTE. Boldface type indicates statistical significance. ART, antiretroviral therapy; BV, bacterial vaginosis; HAART, highly active antiretroviral therapy; Ref, reference; SE, standard error.

^a All factors evaluated but only those with associations where P<0.10 are included in multivariate analysis.

^b Linear regression with generalized estimating equations assuming an exchangeable correlation matrix and identity link is used to estimate β-coefficients, standard errors and P-values.

^c The multivariate models include all evaluated factors where P<0.10 in the univariate model that remained P<0.10 in the multivariate model. Estimates are displayed for all variables that were included in the model.

^d P-trend < 0.001

Table 5
 Association of Demographic, Behavioral, Virologic, and Clinical Factors with HIV-1 Genital Longitudinal Shedding Pattern Among 136 Participants with 3 or More Visits.^a

Factor ^b	HIV-1 genital shedding category ^c			Univariate models ^d			Models adjusted for ART and PVL ^e		
	Never Shedder ^f (N=55)	Intermittent Shedder ^f (N=61)	Persistent Shedder ^f (N=20)	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Antiretroviral therapy									
No therapy	14 (25)	22 (36)	13 (65)	Ref		Ref		Ref	
Mono/Combo	11 (20)	13 (21)	2 (10)	0.42 (0.17–1.05)	0.07	0.63 (0.23–1.67)	0.35		
HAART	30 (55)	26 (43)	5 (25)	0.34 (0.16–0.72)	0.005	0.50 (0.21–1.19)	0.12		
Plasma HIV-1 RNA level, copies/mL									
400	25 (49)	18 (31)	1 (5)	Ref		Ref		Ref	
401–3,999	14 (27)	12 (20)	2 (10)	1.39 (0.55–3.53)	0.48	1.00 (0.36–2.81)	0.99		
4,000–19,999	8 (16)	9 (15)	3 (15)	2.36 (0.82–6.74)	0.11	2.01 (0.69–5.87)	0.20		
20,000	4 (8)	20 (34)	14 (70)	11.65 (4.45–30.53)	<0.001^g	8.87 (3.21–24.54)	<0.001^g		
Unknown	4	2	0						
Per log₁₀ increase									
CD4 cell count, cells/mm ³				2.64 (1.84–3.80)	<0.001	2.47 (1.70–3.60)	<0.001		
0–200	6 (11)	10 (17)	8 (40)	5.67 (2.09–15.37)	0.001^g	2.49 (0.79–7.88)	0.12		
201–350	13 (24)	18 (30)	7 (35)	2.86 (1.24–6.60)	0.01	2.34 (0.92–5.93)	0.07		
351–500	9 (16)	14 (23)	3 (15)	2.41 (0.96–6.01)	0.06	2.58 (0.96–6.90)	0.06		
>500	27 (49)	18 (30)	2 (10)	Ref		Ref			
Unknown	0	1	0						
Alcohol consumption									
Abstainer	31 (56)	32 (53)	5 (25)	Ref		Ref		Ref	
Light (<3 drinks/week)	11 (20)	16 (27)	5 (25)	1.68 (0.76–3.71)	0.20	2.58 (1.05–6.34)	0.04		
Moderate (3–13 drinks/week)	8 (15)	7 (12)	2 (10)	1.04 (0.38–2.87)	0.94	1.10 (0.36–3.32)	0.87		
Heavy (14 or more drinks/week)	5 (9)	5 (8)	8 (40)	4.69 (1.60–13.76)	0.005^h	3.29 (1.10–9.79)	0.03		
Any	24 (43.6)	28 (46.7)	15 (75.0)	1.86 (0.98–3.56)	0.06	2.20 (1.08–4.49)	0.03		
Unknown	0	1	0						

Factor ^b	HIV-1 genital shedding category ^c			Univariate models ^d		Models adjusted for ART and PVL ^e	
	Never Shedder ^f (N=55)	Intermittent Shedder ^f (N=61)	Persistent Shedder ^f (N=20)	OR (95% CI)	P	OR (95% CI)	P
Cervical friability							
Absent	41 (87)	55 (95)	19 (100)	Ref		Ref	
Present	6 (13)	3 (5)	0 (0)	0.26 (0.06–1.04)	0.06	0.20 (0.03–1.20)	0.08
Unknown	8	3	1				
Herpes simplex virus type 2							
Absent	18 (33.3)	14 (24.1)	2 (10.0)	Ref		Ref	
Present	36 (66.7)	44 (75.9)	18 (90.0)	2.13 (1.01–4.52)	0.05	3.21 (1.34–7.67)	0.009
Unknown	1	3	0				
Human papillomavirus, all types							
Absent	25 (61)	23 (49)	2 (15)	Ref		Ref	
Present	16 (39)	24 (51)	11 (85)	2.70 (1.25–5.83)	0.01	2.00 (0.83–4.81)	0.12
Unknown	14	14	7				
Trichomonas vaginalis							
Absent	51 (93)	57 (93)	15 (75)	Ref		Ref	
Present	4 (7)	4 (7)	5 (25)	2.75 (0.87–8.75)	0.09	1.37 (0.38–4.93)	0.63
Summary variable over all visits ^f							
Use of HAART							
Never	12 (22)	14 (23)	7 (35)	Ref		Ref	
Intermittent	21 (38)	36 (59)	12 (60)	1.09 (0.49–2.42)	0.84	1.57 (0.67–3.70)	0.30
Continuous	22 (40)	11 (18)	1 (5)	0.26 (0.10–0.69)	0.006^h	0.46 (0.16–1.30)	0.14
Detectable plasma HIV-1 RNA							
Always	20 (36)	24 (39)	18 (90)	Ref		Ref	
Sometimes	16 (29)	32 (52)	2 (10)	0.54 (0.26–1.11)	0.09	0.48 (0.23–1.02)	0.06
Never	19 (35)	5 (8)	0 (0)	0.09 (0.03–0.27)	<0.001	0.10 (0.03–0.32)	<0.001

NOTE. Data are no. (% of population, unless otherwise indicated. Boldface type indicates statistical significance. ART, antiretroviral therapy; CI, confidence interval; HAART, highly active antiretroviral therapy; Ref, reference.

^aData is restricted to participants with a minimum of 3 and a maximum of 6 evaluated consecutive visits; only at most 1 consecutive visit can be skipped.

^bAll factors were evaluated but only those with associations where $P < 0.10$ in univariate or adjusted models are included in the table.

- ^c Genital shedding is defined as HIV RNA in CVL > 80 copies/mL. Shedding categories are defined as: never shedder, shed at 0 visits; intermittent, shed at 1 or 2 visits; and persistent shedder, shed at 3 or more visits.
- ^d Ordinal logistic regressions are used to estimate odds ratios, 95% confidence intervals and *P*-values where data from the first evaluated visit contributes to the model.
- ^e Adjusted models control for ART category (no therapy, mono/ combo, HAART) and plasma HIV-1 RNA level category (400, 401–3999, 4000–19999, 20000+ copies/mL). The ART model is adjusted for plasma HIV-1 RNA level only, and the plasma HIV-1 RNA level model is adjusted for ART only.
- ^f The adjusted model for use of HAART adjusts only for PVL. The adjusted model for Detectable plasma HIV-1 RNA adjusts only for ART.
- ^g *P*-trend<0.0001;
- ^h *P*-trend<0.01.
- ⁱ Proportionality odds assumption was met for increasing shedding frequency by likelihood ratio test for all variables but Friability and Detectable plasma HIV-1 RNA. These are not reliable due to presence of 0.

Table 6
 Association of Demographic, Behavioral, Virologic, and Clinical Factors with HIV-1 Genital Shedding Frequency Among 62 Women with 3 or More Visits With Detectable Plasma Viral Load.^a

Factor ^b	HIV-1 genital shedding category ^c			Univariate models ^d		Models adjusted for log ₁₀ plasma HIV-1 RNA level ^e	
	Never Shedder (N=20)	Intermittent Shedder (N=24)	Persistent Shedder (N=18)	OR (95% CI)	P	OR (95% CI)	P
Plasma HIV-1 RNA level, copies/mL							
401–3,999	9 (45)	7 (29)	2 (11)	Ref			
4,000–19,999	8 (40)	4 (17)	2 (11)	0.83 (0.21–3.27)	0.79		
20,000	3 (15)	13 (54)	14 (78)	7.28 (2.17–24.39)	0.001^f		
Per log ₁₀ increase				4.09 (1.96–8.55)	<0.001		
CD4 cell count, cells/mm ³							
0–200	7 (35)	6 (26)	2 (11)	3.35 (0.90–12.50)	0.07	1.10 (0.25–4.83)	0.90
201–350	4 (20)	4 (17)	3 (17)	2.49 (0.69–8.97)	0.16	1.73 (0.44–6.83)	0.43
351–500	5 (25)	7 (30)	6 (33)	1.75 (0.41–7.48)	0.45	1.95 (0.44–8.70)	0.38
>500	4 (20)	6 (26)	7 (39)	Ref		Ref	
Unknown	0	1	0				
Alcohol consumption							
Abstainer	14 (70)	11 (46)	5 (28)	Ref		Ref	
Light (<3 drinks/week)	3 (15)	7 (29)	4 (22)	2.53 (0.77–8.25)	0.12	3.96 (1.12–14.02)	0.03
Moderate (3–13 drinks/week)	1 (5)	3 (13)	2 (11)	3.15 (0.63–15.90)	0.16	5.03 (0.75–33.62)	0.10
Heavy (14 or more drinks/week)	2 (10)	3 (13)	7 (39)	6.72 (1.69–26.68)	0.007^f	6.58 (1.56–27.72)	0.01^f
Any	6 (30)	13 (54)	13 (72)	3.62 (1.37–9.58)	0.01	4.92 (1.71–14.14)	0.003
Marijuana/hashish use (past 6 months)							
No	20 (100)	20 (83)	14 (78)	Ref		Ref	
Yes	0 (0)	4 (17)	4 (22)	3.97 (1.00–15.84)	0.05	2.30 (0.53–9.94)	0.26
Squamous metaplasia							
Absent	10 (50)	8 (33)	4 (22)	Ref		Ref	
Present	10 (50)	16 (67)	14 (78)	2.47 (0.92–6.64)	0.07	1.97 (0.70–5.58)	0.20
Candidiasis							

Factor ^{a,b}	HIV-1 genital shedding category ^c			Univariate models ^d			Models adjusted for log ₁₀ plasma HIV-1 RNA level ^e		
	Never Shedder (N=20)	Intermittent Shedder (N=24)	Persistent Shedder (N=18)	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Absent	20 (100)	22 (92)	13 (72)	Ref		Ref		Ref	
Present	0 (0)	2 (8)	5 (28)	9.07 (1.63–50.42)	0.01	15.14 (1.98–116.10)	0.009		
Herpes simplex virus type 2									
Absent	5 (25)	4 (17)	2 (11)	Ref		Ref		Ref	
Present	15 (75)	19 (83)	16 (89)	2.00 (0.59–6.79)	0.27	4.44 (1.10–17.90)	0.04		
Unknown	0	1	0						

NOTE. Data are no. (%) of population, unless otherwise indicated. Boldface type indicates statistical significance. CI, confidence interval; Ref, reference.

^aData is restricted to participants with a minimum of 3 and a maximum of 6 evaluated consecutive visits; only at most 1 consecutive visit can be skipped.

^bAll factors were evaluated but only those with associations where $P < 0.10$ are included in the table.

^cGenital shedding is defined as HIV RNA in CVL > 80 copies/mL. Shedding categories are defined as: never shedder, shed at 0 visits; intermittent, shed at 1 or 2 visits; and persistent shedder, shed at 3 or more visits.

^dOrdinal logistic regressions are used to estimate odds ratios, 95% confidence intervals and P -values where data from the first evaluated visit contributes to the model.

^eAdjusted models control for plasma log₁₀ HIV RNA level.

^f P -trend < 0.01.