Elevated Cervical White Blood Cell Infiltrate Is Associated with Genital HIV Detection in a Longitudinal Cohort of Antiretroviral Therapy–Adherent Women

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Background. Identification of factors associated with the presence of human immunodeficiency virus (HIV) in female genital secretions is critical for intervention strategies targeting transmission and eliminating replication of genital virus. We sought to monitor the prevalence of genital HIV shedding in antiretroviral therapy–adherent women over time and to assess changes in the genital microenvironment.

Methods. Levels of cell-free HIV (HIV RNA) and HIV-infected cells (HIV DNA) were monitored in peripheral blood samples and cervical and vaginal fluid samples at monthly intervals in 11 women for 1 year. Genital tract infections and fluctuations in cervical and vaginal white blood cell counts were also evaluated at each study visit.

Results. Plasma HIV was undetectable at the majority of study visits; when detected, it was only at low levels. Throughout the study, genital HIV RNA and DNA were detected in each person. Combined genital HIV (RNA and DNA) was detected at 49.2% of study visits and was associated with an elevated concentration of cervical white blood cell infiltrate (odds ratio, 2.52 [95% confidence interval, 1.01–6.22]; P = .04). Infiltrate was not associated with a clinical disorder or patient-reported symptoms.

Conclusions. Despite antiretroviral therapy adherence and clinically suppressed plasma viremia, HIV was intermittently detected in genital secretions and was associated with subclinical inflammation and cells trafficking to the cervical mucosa.

The presence of human immunodeficiency virus (HIV) in female genital secretions presents a risk for sexual transmission and mother-to-infant transmission during parturition [1–5]. HIV replication in the genital tract may also increase the risk of pathogenesis because of compartmentalized replication and evolution of virus genotypes [6, 7]. Identification of mechanisms responsible for genital HIV shedding is critical to preventing these adverse events.

Elevated plasma HIV levels and absence of antiretroviral therapy (ART) have been identified as predominant correlates of cervicovaginal HIV detection in several studies [8–12]. Despite strong correlations between plasma viremia and genital shedding, HIV is still detectable in genital secretions in women with low plasma HIV levels, and several studies have documented measurable levels of cervicovaginal HIV in ART-treated cohorts [13–17]. Additionally, differential effects of ART on levels and incidence of genital HIV have been observed. Protease inhibitor–containing ART regimens are more effective in reducing genital HIV levels; however, the steady-state concentration and efficacy of ART in the female genital tract is often drug specific [16, 18, 19]. Understanding of the dynamics of genital HIV
expression in women receiving ART is critical for developing regimens to prevent genital HIV shedding.

Perturbations to the genital microenvironment, such as sexually transmitted infections, genital ulcerations, and vulvovaginal candidiasis (VVC), are also correlated with genital HIV detection [11, 20, 21]. Additionally, other researchers have associated bacterial vaginosis or the absence of Lactobacillus species with increased incidence of genital HIV shedding [8, 22, 23]. Recent studies have also shown a correlation between local inflammatory responses and cytokine levels associated with genital HIV detection [13, 24–26].

Most genital HIV shedding correlates have been derived from cross-sectional studies evaluating a single time point in a dynamic disease course. Only a limited number of studies have longitudinally evaluated genital HIV shedding, and they have similarly associated genital HIV RNA and/or DNA detection over time with higher plasma HIV RNA levels [14, 27, 28]. Other longitudinal studies have evaluated the effect of ART on genital HIV detection, demonstrating a reduction in both plasma and genital HIV levels after initiation of therapy [15, 29]. However, limited longitudinal data are available to describe genital HIV shedding in ART-adherent women, in whom plasma HIV is undetectable or intermittently at a low level [30–32].

In both cross-sectional and longitudinal analyses of cohorts with varying ART use and/or adherence, genital cellular infiltrate has been associated with cervicovaginal HIV RNA detection [13, 33]. These analyses were derived from testing of cervicovaginal lavage fluid, and it is unclear whether the cervix or the vagina was the site of cell trafficking. Nkwanyana et al [26] compared concentration and types of cervical cellular infiltrate between HIV-1–infected women taking ART and those naive to therapy. Infiltrate concentrations were similar in the 2 groups, and data suggested that inflammation at the cervical mucosa was the underlying cause [26]. The relationship between cervical and vaginal infiltrate and genital HIV shedding in ART-adherent women has not been evaluated longitudinally.

Several studies have documented genital HIV shedding in women with low levels of plasma virus [8, 16, 17]. However, few studies have evaluated the prevalence of shedding over time in an ART-adherent cohort or examined correlates of shedding in the absence of plasma viremia. The role played by free virions and infected cells in the transmission or the presence of HIV in the genital mucosa also remains unclear. Chronically infected women adherent to ART regimens who have clinically suppressed viremia present an ideal cohort to evaluate the mechanisms of genital HIV shedding independent of plasma viremia. In light of the widespread and increased use of ART, we sought to assess the prevalence of genital HIV detection in a cohort of ART-adherent women through monthly sampling of both the cervix and the vagina to quantify cell-free and cell-associated HIV levels and to identify associated changes in the genital microenvironment.

**METHODS**

**Population and clinical data.** Eleven women attending a New Orleans HIV clinic were enrolled and evaluated at monthly intervals for 1 year (10 women attended 12 study visits, and 1 woman attended 10 visits). HIV-1–infected, ART-adherent, nonpregnant women ≥18 years old who had an intact cervix were selected. Visits were not conducted during menstruation, and overtly blood-contaminated samples were excluded from analyses. Informed consent was obtained in accordance with the Tulane University Health Sciences Center and Louisiana State University Health Sciences Center Institutional Review Boards.

At each visit, patients completed questionnaires detailing demographic characteristics, date of last menstruation, frequency of vaginal sex and douching, and ART adherence. ART type and CD4 T cell counts were obtained by medical record abstraction.

**Sample collection.** Cervical and vaginal samples were collected on polyester-tipped swabs [13, 16, 34–36] during pelvic examination by a single provider throughout the study. Endocervical secretion samples were obtained by a 360-degree rotation in the os, and vaginal secretion samples were collected by rotating the swab along the vaginal vault. Afterward, a cytobrush (Cooper Surgical) was inserted in the endocervical os to collect cervical cells, placed in Roswell Park Memorial Institute 1640 medium supplemented with gentamycin and amphotericin B, and processed within 4 h of collection [25, 37]. Swab samples collected for genital HIV level analyses were stored in 1 mL of RNAlater solution at 4°C until processed for storage at −80°C, as described elsewhere [35]. Vaginal secretion samples were used to evaluate concomitant genital tract infection. The presence of Trichomonas vaginalis and yeast hyphae and/or conidia were documented by the provider (wet mount). Chlamydia trachomatis and Neisseria gonorrhoeae screenings were conducted at 3-month intervals (GenProbe assay; Becton Dickinson). Bacterial vaginosis diagnoses (Nugent criteria [38]) were performed using Gram-stained vaginal smears. Inflammation of the genital mucosa was assessed during pelvic examination on the basis of the presence or absence of erythematous tissues and excessive or mucopurulent discharge.

Peripheral blood samples were collected in ethylenediaminetetraacetic acid–treated tubes and were fractionated by centrifugation. Plasma aliquots were stored at −80°C; peripheral blood cells (PBCs) were collected from the buffy coat layer, red blood cells were removed by lysis, and cells were stored at −80°C.

**Measurement of HIV levels.** HIV RNA and HIV-infected cell (HIV DNA) levels were quantified using a real-time polymerase chain reaction (PCR) assay targeting the conserved
HIV-1 pol region, as described elsewhere [35]. Virion RNA was purified from 1-mL aliquots of plasma and secretion supernatants. HIV RNA levels were reported as copies per milliliter of plasma or cervical and/or vaginal swab sample. The limit of reproducible quantification was 50 copies per sample. Previous studies indicate that secretion volume does not significantly affect HIV detection [36].

DNA obtained from PBCs and cervical and vaginal cellular fractions (Wizard Genomic kit; Promega) was used for quantifying HIV DNA levels. Using the same set of real-time PCR primers and probes [35], ∼1 µg of DNA was analyzed in each reaction, omitting reverse transcription and using a DNA construct containing the pol target sequence as the quantitation standard. HIV DNA copies were normalized to cell numbers determined by a commercially available RNaseP real-time PCR assay (Applied Biosystems). HIV DNA levels were reported as copies per 1 × 106 cells, and the limit of detection was 1 copy per 1 × 103 cells.

Longitudinal PBC profile. Longitudinal fluctuations of HIV-infected PBC levels were characterized as stable or variable. PBC levels measured over time in each patient were plotted, and the standard error of points along the slope were determined. Stability or variability was based on the significance of slope standard error according to the t statistic test (significance indicated variability, and nonsignificance indicated stability).

Cell population analysis. Cervical cell types were enumerated by microscopic examination using Endtz-trypsin stain to differentiate epithelial cells, polymorphonuclear cells (PMNs), and mononuclear cells; to compute the total white blood cell (WBC) count; and to evaluate red blood cell (RBC) counts [39]. Cervical cells were enumerated from a suspension of cells eluted from the cytobrush, and ≥100 cells were counted, increasing the sensitivity of cell-type characterization, compared with ≥30 PMNs per high-powered field measures [8, 40, 41], similar to methods described by others [33]. Total WBC counts were enumerated in the vaginal mucosa by means of Gram-stained vaginal smears.

Each cervical cell type was normalized as a percentage of the total cell population. Elevated numbers of each WBC type were defined on the basis of the distribution obtained from the entire cohort. Elevated numbers of each cell type were defined as those in the fourth quartile of the cohort’s values. Samples with cell-type percentages exceeding the median value that determined the fourth quartile were considered to be elevated. Elevated numbers of RBCs were designated as >2.5 × 105 cells/mL of cell suspension.

The same rubric for defining elevated numbers of vaginal WBCs was used to maintain consistency in analyses. In these samples, “elevated” designations were compared between the methods of >30 PMNs per high-powered field and distribution of percentages. The number of time points with elevated vaginal WBC counts differed by only 1 between the 2 approaches.

Table 1. Baseline Demographics (n = 11)
Figure 1. Longitudinal human immunodeficiency virus (HIV) RNA and DNA levels in 11 women. Levels were measured at 12 study visits in 10 patients and at 10 study visits in patient 6. Cervical levels are indicated by black circles, and vaginal levels are represented by white triangles; plasma (RNA) and peripheral blood cell (PBC; DNA) levels are indicated by white squares. A, HIV RNA levels over the course of the study. The limit of quantification was 50 copies/cervical or vaginal swab specimen or milliliter of plasma, as indicated by the dotted line. One value (high-level blip; patient 6) lay outside the plot (copies/mL) and is indicated by a dagger (†). The mean genital HIV RNA level was 102 copies/mL, and the mean vaginal HIV RNA level was 115 copies/mL. The mean cervical HIV RNA level was 50 copies/mL, and the mean plasma HIV level, including the high-level blip, was 2226 copies/mL (50 copies/mL excluding the high-level blip). B, HIV DNA levels over the course of the study. The limit of detection was 1 copy/10^5 cells. One value lay outside the plot (‡); the corresponding HIV DNA level is 1190.5 copies/10^5 cells. The mean genital HIV DNA level was 73 copies/10^5 cells, and the mean vaginal HIV DNA level was 102 copies/10^5 cell. The mean cervical HIV DNA level was 47 copies/10^5 cells, and the mean PBC HIV DNA level was 65 copies/10^5 cells.

Statistical analyses. Generalized estimating equations were used to evaluate longitudinal associations of genital HIV detection and concomitant factors and to accommodate the repeat-measure design. Statistical significance was defined as P < .05 for unadjusted and multivariate analyses. Univariate associations were evaluated by the χ² test. The t statistic test was used to determine the significance of slope standard error when evaluating longitudinal HIV-infected PBC levels.

RESULTS

Peripheral and genital HIV detection. HIV levels were monitored on a monthly basis in a cohort of 11 women receiving combination ART regimens (Table 1 and Table 2). Peripheral HIV levels were measured in plasma (HIV RNA) and PBCs (HIV DNA) at each visit. Over the course of the study, plasma HIV RNA was detectable at only 20% of the study visits. When detectable, plasma HIV levels were low (50–152 copies/mL), with the exception of a single blip (2.9 × 10^5 copies/mL) in 1 patient (patient 6) (Figure 1A). Thus, these women were classified as having clinically suppressed viremia. Levels of HIV DNA in PBCs ranged from undetectable to 483 copies/10^5 cells (Figure 1B). The majority of women (n = 9) had persistently detectable levels of HIV DNA in PBCs.

Despite low levels of HIV in peripheral blood, quantifiable levels of HIV were found in the genital tract over the course of the study (Figure 1A and 1B). Detectable vaginal and cervical HIV RNA levels ranged from 50 to 525 HIV RNA copies/swab specimen; HIV RNA levels in vaginal secretions were slightly higher than those in cervical secretions. Genital HIV DNA levels ranged from 2.4 to 1200 copies/10^5 cells.

The frequency of HIV RNA and DNA detection in blood, cervical, and vaginal samples was evaluated as the rate per study visit for each patient (Figure 2). Similar frequencies of HIV RNA detection were observed in the cervix and vagina; however, concordance (detection in both compartments) was limited, occurring only 3 times in 3 patient (10.7%; P = .40, χ² test).
Figure 2. Rate of human immunodeficiency virus (HIV) detection per study visit among patients. The number of study visits at which genital HIV RNA (cervical and/or vaginal fluids), genital HIV DNA (cervical and/or vaginal cellular fractions), combined genital HIV (genital HIV RNA and/or DNA), and plasma HIV RNA were detected. HIV was monitored at 12 study visits for all patients except patient 6, for whom analyses were conducted for 10 study visits.

When vaginal and cervical HIV RNA detection observations were evaluated together (termed genital HIV RNA), the rate of genital HIV RNA detection was slightly increased, compared with plasma detection rates (23.1% vs 20%) (Table 3).

Cervical and vaginal HIV DNA detection rates were also grouped (termed genital HIV DNA), and genital HIV DNA was more frequently detected than was genital HIV RNA. A similar low-level concordance was observed between the cervix and vagina for HIV DNA detection (4 times in 3 patients; 11.1%; $P > .99, \chi^2$ test). Despite undetectable to low-level plasma viremia, both genital HIV RNA and genital HIV DNA were intermittently detected in each patient over time.

Concordant HIV RNA detection in vaginal and cervical compartments occurred in patients different from those in whom concordant detection of genital HIV DNA occurred. These data suggest that the presence of HIV in cervical and vaginal secretions and/or cell populations is independent of virus present in the other genital compartment.

Detections of genital HIV RNA (cell-free virions) and genital HIV DNA (infected cells) were also combined (termed genital HIV) to evaluate the likelihood of detecting any genital HIV at a single study visit. Genital HIV was detected at 49.2% of study visits. In 2 patients (patients 8 and 13), genital HIV was detected at $\geqslant 50\%$ of study visits, despite persistently undetectable levels in plasma.

Genital HIV detection was not associated with detection of HIV in plasma ($P = .30$ and $P = .51$ for genital HIV RNA and DNA, respectively; $\chi^2$ test). Only 26.7% of genital HIV RNA-positive time points and 16.7% of genital HIV DNA–positive time points coincided with plasma HIV detection. Because of the paucity of data describing longitudinal fluctuations of HIV-infected PBCs in chronically infected ART-adherent women, HIV levels in these cells over time were characterized as stable or variable (Table 4). Only 3 of 12 women were characterized as having variable levels of HIV-infected PBCs over time. Neither stable nor variable levels of HIV-infected PBCs were associated with the presence or absence of genital HIV RNA or DNA by generalized estimating equation analyses.

Genital tract infections. A low prevalence of genital tract infection was observed. The presence of yeast hyphae and/or conidia was recorded at 7 of 127 study visits but was not indicative of VVC. VVC symptoms (ie, itching, curdlike discharge, and the presence of PMNs) were not documented concomitant with hyphae and/or conidia observations. Despite the low frequency, the presence of yeast hyphae and/or conidia in vaginal secretions was associated with a 1.8-fold increased risk of genital HIV detection (95% confidence interval [CI], 1.15–6.32; $P = .02$). Erythematous cervicovaginal tissue and excessive and/or mucopurulent discharge were observed at frequencies of 0.77% (1/130 visits) and 8.4% (8/130 visits), respectively.

Genital sexually transmitted infections did not affect HIV detection. Results of all C. trachomatis and N. gonorrhoeae tests were negative. T. vaginalis was detected in 1 patient at a single visit. Herpesvirus lesions were not documented by the provider at any study visit. Although bacterial vaginosis was associated with genital HIV detection in previous cross-sectional studies [11, 20, 23], an association was not observed in this cohort, despite a prevalence of 40.2%.

Genital cellular populations. Cervical PMN and mononuclear cell concentrations were first evaluated independently and then analyzed together as total cervical WBC infiltrate. In the vaginal compartment, total WBC infiltrate was analyzed. Elevated cervical cellular infiltrate concentration was determined by fourth-quartile distribution of the entire cohort’s
cervical cell concentrations (Figure 3). Patients 2, 5, 6, 19, and 21 had consistently elevated concentrations of ≥1 cell type in the genital compartment (Figure 4). Elevated numbers of cervical PMNs were observed less frequently than elevated cervical mononuclear cells. Seven women had time points with elevated total cervical WBC counts; cervical WBC counts were consistently elevated in patients 5, 6, 8, and 19. Coincident infection, inflammation, or patient-reported symptoms were not associated with higher numbers of PMNs, mononuclear cells, and/or WBCs in the cervix. An elevated concentration of cervical WBCs was associated with a 2.61-fold higher risk of genital HIV detection (95% CI, 1.01–6.22; P = .04). Elevated numbers of vaginal WBCs were observed in only 3 women, and patients 5 and 21 had elevated vaginal leukocyte numbers at 90% and 91.7% of study visits, respectively (Figure 4).

Elevated numbers of RBCs were observed at least once in 10 of 11 patients. Blood was not visible before cytobrush sample collection, and overt blood contamination was not observed in matched cervical swab specimens collected before brush samples. Therefore, RBCs were elicited by gentle abrasion during cytobrush sample collection. In unadjusted analyses, the presence of RBCs was associated with genital HIV DNA and combined genital HIV detection (P = .01 and P = .03, respectively) (Table 5), suggesting that a compromised or friable cervical mucosa may underlie the presence of HIV in genital secretions. An association was not observed in multivariate analyses.

### DISCUSSION

The present study details the prevalence of HIV in genital secretions from women with clinically suppressed plasma viremia.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Value</th>
<th>SE</th>
<th>t</th>
<th>P</th>
<th>95% CI</th>
<th>Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−0.22</td>
<td>0.09</td>
<td>2.45</td>
<td>.03</td>
<td>0.02 to 0.43</td>
<td>Variable</td>
</tr>
<tr>
<td>2</td>
<td>0.15</td>
<td>0.22</td>
<td>0.67</td>
<td>.52</td>
<td>−0.35 to 0.65</td>
<td>Stable</td>
</tr>
<tr>
<td>3</td>
<td>−0.15</td>
<td>0.06</td>
<td>−2.52</td>
<td>.03</td>
<td>−0.28 to −0.02</td>
<td>Variable</td>
</tr>
<tr>
<td>4</td>
<td>−0.14</td>
<td>0.13</td>
<td>−0.98</td>
<td>.34</td>
<td>−0.44 to 0.17</td>
<td>Stable</td>
</tr>
<tr>
<td>5</td>
<td>−0.34</td>
<td>0.33</td>
<td>−1.04</td>
<td>.32</td>
<td>1.10 to 0.42</td>
<td>Stable</td>
</tr>
<tr>
<td>6</td>
<td>−0.04</td>
<td>0.02</td>
<td>−2.04</td>
<td>.07</td>
<td>−0.08 to 0.003</td>
<td>Stable</td>
</tr>
<tr>
<td>7</td>
<td>0.10</td>
<td>0.03</td>
<td>3.46</td>
<td>.006</td>
<td>0.03 to 0.16</td>
<td>Variable</td>
</tr>
<tr>
<td>8</td>
<td>1.22</td>
<td>3.00</td>
<td>0.41</td>
<td>.69</td>
<td>−5.46 to 7.91</td>
<td>Stable</td>
</tr>
<tr>
<td>9</td>
<td>0.02</td>
<td>0.15</td>
<td>0.11</td>
<td>.91</td>
<td>−0.31 to 0.34</td>
<td>Stable</td>
</tr>
<tr>
<td>10</td>
<td>0.01</td>
<td>0.07</td>
<td>0.18</td>
<td>.86</td>
<td>−0.15 to 0.18</td>
<td>Stable</td>
</tr>
<tr>
<td>11</td>
<td>0.13</td>
<td>0.42</td>
<td>0.31</td>
<td>.76</td>
<td>−0.81 to 1.09</td>
<td>Stable</td>
</tr>
</tbody>
</table>

**NOTE.** Longitudinal HIV-infected PBC levels were plotted on the y-axis, and the slope and standard error (SE) of points along the slope were determined. The significance of the SE (P<.05) was determined by the t statistic test and 95% confidence intervals (CI), excluding zero. A significant SE, indicative of HIV-infected PBC levels significantly deviating from the slope, resulted in a variable designation to describe longitudinal HIV-infected PBC levels; a nonsignificant SE indicated stable levels of HIV-infected PBCs over time.
Figure 3. Percentage distribution of types of genital cellular infiltrate. Cell types were enumerated from cervical cytobrush samples (n = 130) and Gram-stained vaginal secretion smear samples (n = 127). Cell number was normalized to percentage (y-axis). Percentages of each cell type from all times when samples were performed are plotted on the x-axis. The distribution of percentages was analyzed to determine the fourth-quartile distribution for each white blood cell (WBC) type. Percentages, or concentrations, of cells in the fourth quartile were deemed to be elevated and are identified by black text boxes. A, Cervical mononuclear cells (elevated, >48.2%). B, Cervical polymorphonuclear cells (PMNs) (elevated, >24.4%). C, Cervical WBCs (elevated, >78.2%). D, Vaginal WBCs (elevated, >35.7%).

and self-reported ART adherence. By using this cohort, HIV-shedding correlates and potential genital tract viral reservoirs could be evaluated in the absence of plasma viremia. Despite undetectable to low levels of plasma HIV, genital HIV was detected at 49.2% of study visits and was not associated with intermittent plasma virus detection. These data are similar to those in a recent longitudinal study of seminal plasma in ART-adherent men, in which genital HIV was detected at 48% of study visits [42]. Elevated numbers of cervical PMNs, mononuclear cells, and/or total cervical WBCs were observed in each patient over time, and elevated numbers of cervical WBCs were associated with an increased risk of genital HIV detection (odds ratio, 2.61 [95% CI, 1.01–6.22]; P = .04). However, cellular infiltrate was not associated with a concomitant infection or disorder. These analyses demonstrate that HIV is frequently present in female genital secretions despite ART use and controlled viremia and that detection of genital virus is likely to be attributable to subclinical fluctuations in the genital microenvironment and trafficking of cells to the mucosa.

Using this longitudinal study design, we systematically evaluated the prevalence of cervical and vaginal cell-free virions and HIV-infected cells. Vaginal and cervical HIV RNA and DNA detection was combined to generate statistical power to elucidate mechanisms associated with genital HIV. Despite a small sample size, we were able to associate increased cervical cellular infiltrates with genital HIV shedding over time. Additionally, we described a novel, longitudinal characterization of cervical and vaginal cell populations that may benefit topical microbicide strategies targeted in the female genital mucosa.

The presence of virus in vaginal secretions suggests potential viral replication at a site with unique immune and environmental pressures. Although only low-level genital HIV was observed intermittently in each patient, the viral levels necessary to pose a risk for transmission and host pathogenesis are un-
known, and low levels could readily increase during temporary
interruptions of ART. The longitudinal effect of ART interrup-
tions on genital virus shedding remains to be studied. Only 3
women reported a brief therapy interruption the day before
the study visit; however, a higher frequency of ART interrup-
tion is probable in studies with larger populations. In this context,
it is important to understand the mechanisms of even low-
level genital HIV shedding.

The presence of yeast hyphae and/or conidia was associated
with genital HIV detection in multivariate analyses but was not
concomitant with VVC symptoms. Additionally, strains were
not speciated; thus, it is possible that the strain often implicated
in VVC, Candida albicans, was not present. These findings war-
rant additional study in larger cohorts.

Previous studies have associated cervicovaginal HIV detec-
tion with menstrual cycle phase [34, 43, 44]. In the present
study, samples were collected at consistent time points to en-
sure that menstrual cycle changes could not contribute to flu-
cuation in genital HIV level in each woman. An unadjusted
univariate association was observed between genital HIV detec-
tion and menstruation regularity, but associations were not
observed with cycle phase.

Patients with elevated cervical WBC counts were 2.61 times
more likely to have detectable levels of genital HIV. These data

Table 5. Factors Associated with Genital Human Immunodeficiency Virus (HIV) Detection in a Longitudinal Cohort
of Women with Controlled Viremia Who Were Adherent to Antiretroviral Therapy (n = 11; 130 Observations)

<table>
<thead>
<tr>
<th>Univariate predictor (unadjusted GEE)</th>
<th>Genital HIV-1 RNA detection</th>
<th>Genital HIV-1 DNA detection</th>
<th>Combined Genital HIV (RNA and/or DNA) detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P&lt;sup&gt;a&lt;/sup&gt;</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>CD4 T cell count &lt;400 cells/µL</td>
<td>0.49 (0.24–1.00)</td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>Regular vs irregular menstruation&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.27 (0.12–0.62)</td>
<td>.002</td>
<td>0.56 (0.34–0.91)</td>
</tr>
<tr>
<td>Vaginal sex exposure&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.44 (0.20–0.94)</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>Elevated cervical WBC count&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td>2.98 (1.33–6.64)</td>
<td>.01</td>
<td>2.61 (1.34–5.10)</td>
</tr>
<tr>
<td>Presence of cervical RBCs&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.79 (1.32–5.92)</td>
<td>.01</td>
<td>2.27 (1.09–4.71)</td>
</tr>
<tr>
<td>Presence of yeast&lt;sup&gt;e,g&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>1.80 (0.96–3.34)</td>
</tr>
</tbody>
</table>

**NOTE.** GEE, generalized estimating equation; HIV-1, HIV type 1; RBC, red blood cell; WBC, white blood cell.

<sup>a</sup> GEE unadjusted P values.
<sup>b</sup> Determined on the basis of the self-reported date of the last day of menstruation.
<sup>c</sup> Self-reported frequency (any) in the previous month.
<sup>d</sup> Elevated cervical WBC count was considered to be 78.2%, as determined by enumeration of stained, cervical brush–collected cell populations.
<sup>e</sup> Significant in multivariate, adjusted GEE analyses.
<sup>f</sup> Moderate (26–75) to high (≥75) nos. of RBCs enumerated from cervical brush–collected cell populations.
<sup>g</sup> Presence of yeast hyphae and/or conidia in vaginal secretions, as determined from Gram-stained smears.
<sup>h</sup> Presence of yeast was nonsignificant in univariate analyses, but significance was observed in multivariate logistic regression.
corroborate those of Anderson et al [33], which were derived from a cohort with mixed ART use by testing cervicovaginal lavage samples for extended follow-up periods. Higher concentrations of cervical cell infiltrates in HIV-infected women with local inflammation have been observed previously [26]. In our analyses, cervical infiltrate was associated with genital HIV detection and was frequently observed in the absence of infection or patient-reported symptoms, although we were unable to assess other inflammatory markers described elsewhere [26]. Additionally, elevated cervical RBC counts were associated with genital HIV detection ($P = .01$ and $P = .03$ for DNA and RNA/DNA detection, respectively, in unadjusted univariate analyses), suggesting that a friable cervical mucosa may also be a factor in shedding. Friability could facilitate HIV target cell migration or plasma transudate leakage. WBCs migrating through cervical tissue could introduce breaks through which erythrocytes could also pass or leak whole blood containing WBCs and RBCs. However, only a 29.7% concordance rate was observed between elevated cervical WBC and RBC counts. RBCs were not considered to be contaminants and were elucidated only at obtainment of cytobrush samples. Other researchers have determined that RBCs have a negligible effect on endocervical leukocyte populations and likely constitutively contribute to mucosal dynamics [45]. Mucosal friability and subclinical inflammation could be attributed to age (mean, 46.8 years), but subclinical inflammation has been described over a wide age range [22, 24, 25]. Regardless, friable cervices were noted in this cohort, coincident with genital HIV detection at almost half the study visits; thus, a risk of transmission is plausible, particularly if additional abrasion to the genital mucosa is introduced through vaginal intercourse.

These data underscore the public health relevance and need to continually educate women of existing transmission risk, despite ART adherence and undetectable plasma HIV-1 viral load. Genital virus exposure independent of plasma viral load is frequently observed in the absence of infection or patient-reported symptoms, although we were unable to assess other inflammatory markers described elsewhere [26]. Additionally, elevated cervical RBC counts were associated with genital HIV detection ($P = .01$ and $P = .03$ for DNA and RNA/DNA detection, respectively, in unadjusted univariate analyses), suggesting that a friable cervical mucosa may also be a factor in shedding. Friability could facilitate HIV target cell migration or plasma transudate leakage. WBCs migrating through cervical tissue could introduce breaks through which erythrocytes could also pass or leak whole blood containing WBCs and RBCs. However, only a 29.7% concordance rate was observed between elevated cervical WBC and RBC counts. RBCs were not considered to be contaminants and were elucidated only at obtainment of cytobrush samples. Other researchers have determined that RBCs have a negligible effect on endocervical leukocyte populations and likely constitutively contribute to mucosal dynamics [45]. Mucosal friability and subclinical inflammation could be attributed to age (mean, 46.8 years), but subclinical inflammation has been described over a wide age range [22, 24, 25]. Regardless, friable cervices were noted in this cohort, coincident with genital HIV detection at almost half the study visits; thus, a risk of transmission is plausible, particularly if additional abrasion to the genital mucosa is introduced through vaginal intercourse.

These data underscore the public health relevance and need to continually educate women of existing transmission risk, despite ART adherence and undetectable plasma HIV. Furthermore, these studies highlight the need for diligent disease status monitoring and use of appropriate therapy regimens, even in patients responding well to therapy. Genital virus exposure, particularly in the context of a therapy interruption, may propagate a unique or drug-resistant variant with the capacity to drive host pathogenesis.

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