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Association of Cervical Biopsy with HIV Type 1 Genital Shedding Among Women on Highly Active Antiretroviral Therapy

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Abstract

HIV-1 genital shedding is associated with increased HIV-1 transmission risk. Inflammation and ulceration are associated with increased shedding, while highly active antiretroviral therapy (HAART) has been shown to have a protective effect. We sought to examine the impact of cervical biopsies, a routine component of cervical cancer screening, on HIV-1 genital RNA levels in HIV-infected women on HAART. We enrolled HIV-1-infected women undergoing cervical biopsy for diagnosis of cervical intraepithelial neoplasia (CIN) 2/3 in this prospective cohort study. All were stable on HAART for at least 3 months. Clinical and demographic information as well as plasma HIV-1 viral load were collected at the baseline visit. Specimens for cervical HIV-1 RNA were collected immediately prior to biopsy, and 2 and 7 days afterward. Quantitative PCR determined HIV-1 concentration in cervical specimens at each time point to a lower limit of detection of 40 copies/specimen. Among the 30 participants, five (16.6%) women had detectable cervical HIV-1 RNA at baseline, of whom four (80%) had detectable HIV-1 RNA after cervical biopsy, with no significant increase in viral load in the follow-up specimens. Only one woman (3.3%) with undetectable baseline cervical HIV-1 RNA had detection postbiopsy. Detectable plasma HIV-1 RNA was the only factor associated with baseline cervical HIV-1 RNA. In women on HAART, an increase in cervical HIV-1 RNA detection or concentration was not associated with cervical biopsy. These findings help provide safety data regarding cervical cancer screening and diagnosis in HIV-infected women and inform postprocedure counseling.

Introduction

Cervical cancer is the second most common cancer among women in sub-Saharan Africa.[1] This region also bears the largest HIV burden in the world.[2] As more resource-limited countries develop the infrastructure to provide clinical care and antiretroviral therapy to reduce the immediate mortality risk of AIDS, effective cervical cancer screening programs that address the unique biological relationship between HIV, cervical intraepithelial neoplasia (CIN), and cervical cancer are needed. Among the challenges faced by these programs is determining the safety of screening, diagnostic, and treatment methods in an HIV-1-positive population. Although a recent study showed that diagnostic and treatment procedures did not increase the risk of HIV acquisition among HIV-negative women,[3] few studies have evaluated the potential impact that screening methods could have on sexual transmission of HIV-1.

Sexual transmission of HIV-1 has been associated with the presence and quantity of HIV-1 in the genital tract.[4] Studies examining women with cervical and genital ulceration, inflammation, or concomitant sexually transmitted infections (STIs) demonstrated increased levels of HIV-1 RNA in cervicovaginal secretions, referred to as increased HIV-1 genital shedding.[5–10] One small study showed increased HIV-1 RNA genital shedding after various treatments for CIN.[11] Diagnostic procedures such as cervical biopsy also denude the cervical mucosa and cause inflammation, and therefore they could alter the concentration of genital HIV-1 RNA and possibly increase HIV-1 transmission risk.

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In the United States, one study has shown that 75% of women with HIV-1 undergo cervical cancer screening,[12] and each woman has the potential to undergo multiple biopsies in her lifetime. In resource-limited settings, the number of women currently undergoing screening is small, but with growing interest and resources to provide cervical cancer screening, it is anticipated that the number of women able to undergo screening will vastly increase. While currently the majority of women in resource-limited settings with access to services undergo screening and same-visit treatment ("see and treat" strategies), the use of cervical biopsy has been shown to increase specificity and reduce rates of overtreatment. As programs become more sophisticated in their ability to offer comprehensive screening, biopsies may become increasingly common. Thus, there is the potential to have a large population of HIV-1-infected women undergoing screening procedures, with relatively little knowledge of the effect this intervention could have on cervical shedding levels and HIV-1 transmission.

Several studies have shown that highly active antiretroviral therapy (HAART) reduces HIV-1 RNA in genital secretions[13–15] and limits the increase in HIV-1 shedding after cervical infection.[16] Based on this observed protective effect of HAART on HIV-1 RNA in genital secretions, along with the increase in availability of HAART and the likely pairing of cervical cancer screening programs with HIV care including HAART provision, we sought to examine the durability of the protective effect of HAART on HIV-1 genital shedding following cervical biopsy. This study examines the impact of performing a colposcopically directed cervical biopsy on HIV-1 RNA in genital secretions in HIV-1-infected women receiving HAART.

Materials and Methods

Study participants

This study was one component of a large prospective cohort trial to examine the safety and efficacy of various cervical cancer screening techniques among HIV-1-infected women. The aim of this study was to compare genital HIV-1 RNA detection and concentration before colposcopically directed biopsy and then 2 and 7 days after biopsy. This follow-up period was chosen because although there are no standard guidelines on counseling for postprocedure abstinence, most clinicians recommend 2–3 days, or until vaginal bleeding/spotting subsides. Human subjects approval was received from all collaborating institutions: Stanford University School of Medicine, University of California, San Francisco, and the Kenya Medical Research Institute, and written informed consent was obtained prior to study enrollment.

Between October 2010 and March 2011, HIV-1-infected women who underwent colposcopically indicated biopsy for suspected CIN 2/3 at the Family AIDS Care and Education Services (FACES) clinic in Kisumu, Kenya, were invited to enroll in this component of the study. They were eligible to be enrolled if they were not pregnant, at least 23 years of age (age screening is initiated at FACES), had been adherent on HAART for at least 3 months with no clinical or immunologic evidence of treatment failure, and had no clinical evidence of STIs including genital ulcers, candidiasis, cervicitis or wet mount evidence of *Trichomonas vaginalis*. We chose the HAART and STI inclusion criteria based on the strong effect both of these have shown on HIV-1 genital shedding.[17] All participants were on the standard HAART regimens available at FACES, per the Kenyan Ministry of Health Guidelines. Women were excluded from participation if they reported intercourse in the past 3 days, to reduce the potential contribution of seminal fluid to HIV-1 detection.

As part of the routine clinical care, baseline clinical and demographic variables were collected for each woman at the time of the procedure, including age, $CD4^+$ count, WHO stage, HAART regimen and duration, hormonal contraception use, gravidity, parity, and number of current and lifetime sexual partners. Women who underwent a cervical biopsy were asked to return for follow-up visits 2 and 7 days after their procedure for repeat cervical specimen collection and a short questionnaire assessing symptoms and sexual activity. A visual examination of the cervix to assess for healing was also performed at each visit. As the impact of CIN itself on shedding is unclear,[18] we choose to focus our analysis on CIN 2/3, at this would be the most likely indication for cervical biopsy in a general population and as it is the most severe lesion and would most likely be associated with HIV-1 genital shedding. Final study eligibility was determined after biopsy results confirmed presence of CIN 2/3 and laboratory results were negative for *Trichomonas vaginalis, Chlamydia trachomatis or Neisseria gonorrhoeae*. We completed study enrollment once we had recruited 30 women who fulfilled our study criteria.

Cervical HIV-1 viral load specimen collection and measurement

Each participant had a pelvic examination with visual inspection with acetic acid (VIA) as part of the cervical cancer screening. Immediately after speculum insertion, a trained study nurse or clinical officer inserted three Tear Flo strips together (Odyssey Medical, Bartlett, TN) into the cervical os. Absorbed strips were withdrawn, cut at the 15 mm line, and submerged in 1.0 ml Abbott lysis buffer solution. Each wick is estimated to collect approximately 12 μl of fluid, for a total of 36 μl collected using three wicks per participant. Specimens were stored on-site at FACES at -80°C for up to 4 months before being shipped on dry ice to the ARI-UCSF Laboratory of Clinical Virology for HIV-1 RNA testing using the Abbott RealTime Viral Load assay.

After tear flo strips were collected, VIA was performed followed by colposcopy. Participants who were suspected of having high-grade dysplasia by colposcopy examination had cervical biopsy samples taken. Biopsies were taken with biopsy forceps by the clinical officer and placed in formalin. The location, size, and number of biopsies (1 or 2) were recorded and later transcribed to a coded study research document. Biopsies were then sent to a pathologist in Nairobi who graded them. Only participants found to have CIN 2/3 had evaluation of their Tear Flo strips.

HIV-1 viral load measurements from cervical wicks used the 0.6 ml protocol with a LLOD of 40 copies per specimen (cps) at 95% probability[19] and measurements are reported as RNA cps collected from three combined Tear Flo strips. Women who were found to have detectable cervical viral load at any point (baseline, day 2 after biopsy, and day 7 after biopsy) were defined as "shedders" while women who never displayed detectable genital shedding were defined as "non-shedders."

Blood plasma viral load collection and measurement

Participants who had cervical HIV-1 RNA samples collected also had blood samples collected at the same initial study enrollment visit in order to measure plasma HIV-1 RNA. Specimens were immediately processed at the FACES on-site laboratory following standard procedures. The plasma was stored up to 3 months on-site at FACES at −80°C before being shipped on dry ice for HIV-1 RNA measurement in Nairobi. HIV-1 RNA measurements were determined using the Abbott RealTime Viral Load assay, with a lower limit of detection (LLOD) of 40 copies per sample (Abbott Molecular, Des Plaines, IL).

Statistical analysis

We determined a priori that a sample size of 30 women would be sufficient to show a significant difference between baseline and postbiopsy HIV-1 RNA detection and concentration in genital secretions within woman. We estimated that among women on HAART, approximately 20% would have detectable HIV in their genital secretions.[20] We based our effect size on data from a previous study that examined women with CIN before and after treatment[11]; women were found to have a 1.0–4.4 $\log_{10}$ increase (mean of 2.3 log increase) in HIV-1 RNA copies 2 weeks after treatment. Based on this finding, we powered our study to detect at least a 1.2 $\log_{10}$ increase. This was chosen as an appropriate cut-off as it reflects the minimal amount of change seen in the previous studies. Since our levels were measured closer to the biopsy date (day 2 and day 7) than the previous study (week 2 and week 4), we expected to see a higher level of change than what was previously observed. All statistics, including means, medians, and ranges, as well as univariate analyses for association of baseline characteristics with genital HIV-1 shedding on baseline visit with Student's *t*-tests were calculated using STATA 11 (StataCorp LP, College Station, Texas).

Results

Between October 2010 and April 2011, 75 women on HAART underwent cervical biopsy as part of a cervical cancer screening and were confirmed to have CIN 2/3. Of these 75 women, 43 were not eligible for study enrollment. The majority of the women excluded ($n=23$, 51.1%) had not been on their current HAART regimen for greater than 3 months and so did not qualify as being on a stable regimen. Seventeen (37.8%) did not return for both follow-up visits and three (6.7%) were found to have a sexually transmitted infection. Two (4.4%) had results that were reported after enrollment was complete. Of the 30 women who were included in the analysis, the mean age was 31.7 years ± 7.2 years, mean $CD4^+$ count was 374/μl ± 349/μl, mean duration on HAART was 19.2 months ± 21.7 months, and mean lifetime number of sexual partners was 4 ± 2.1 (Table 1). Baseline clinical and demographic characteristics did not differ between these women and the women who were excluded from the study.

Twenty-four (80%) participants had undetectable HIV-1 RNA in genital secretions at all visits ("nonshedders"). Six women (20%) had detectable HIV-1 RNA in genital secretions at one or more of the time points ("shedders"), of whom five (16.7%) had detectable genital shedding at baseline. There was no association between baseline clinical and demographic characteristics such as $CD4^+$ T cell count, HAART duration, HAART regimen, and hormonal contraception use with the detection of cervical HIV-1 RNA (Table 1). There was no difference in visual appearance of healing for women after biopsy who displayed shedding compared to those who did not at either time point.

The only woman with postbiopsy shedding after undetectable baseline cervical HIV-1 RNA had 183 cps at day 2 and was undetectable again at day 7. Four women (13.3%) had detectable cervical HIV-1 RNA at baseline and at least one postbiopsy visit (Table 2). There was no statistically significant increase in HIV-1 RNA concentration in genital secretions after biopsy in any of the women with detectable baseline HIV-1 RNA. Although all four women with shedding at baseline and after biopsy had a peripheral blood $CD4^+$ T cell count below 250 cells/μl, the mean $CD4^+$ count was not significantly different between shedders and nonshedders.

Detectable blood plasma HIV-1 RNA was associated with baseline HIV-1 RNA in genital secretions; the mean blood plasma HIV-1 RNA concentration for all participants was 12,323 copies/sample for genital shedders vs. 97.4 copies/sample for genital nonshedders ($p<0.05$). Among nonshedders, 75% (18/24) had suppressed baseline blood plasma HIV-1 RNA (<40 copies/ml) versus 33% (2/6) of the shedders. Among women who had detectable plasma viral load compared to those who did not, the proportion of genital shedders was not different (25% vs. 18%, chi square = 0.17, $p=0.68$).

Discussion

Among women stable on HAART for at least 3 months, cervical HIV-1 RNA detection was rare, and the concentration did not increase at 2 and 7 days following cervical biopsy. Cervical HIV-1 RNA was undetectable in 83% of participants. This proportion is similar to previous reports, which showed that up to 85% of women on HAART no longer had detectable HIV-1 RNA in genital secretions.[14,17] Our findings demonstrated that even after the ulceration and inflammation resulting from the cervical biopsy, detection and concentration of HIV-1 RNA genital shedding remained unchanged in women with CIN 2/3 on HAART. This supports the safety of this procedure in HIV-1-infected women who are on HAART. These reassuring findings provide useful safety information for providers taking care of HIV-infected women in any setting. They are especially important in settings in which women are not always able to negotiate sex with their partners, impacting their ability to remain abstinent after the procedure.[21]

Of the six women who did have detectable HIV-1 genital shedding, only four had detectable genital HIV-1 viral load both at baseline and after biopsy. There was a statistical difference in plasma viral load between women who displayed genital shedding and women who did not. However, on closer inspection of the data, it was noted that this finding was primarily offset by one participant who had a significantly elevated plasma viral load. When this person was excluded, no relationship was seen between detectable plasma viral load, and baseline genital shedding. We did not find a statistical relationship between $CD4^+$ count, HAART duration, or type of HAART regimen with the detection of HIV-1 RNA genital shedding. Although this study was powered to look at differences in detection and concentration between baseline

Table 1. Baseline Characteristics of Study Participants Who Displayed HIV-1 Genital Shedding ($n=6$) at Any Study Visit Compared to Participants with No Shedding ($n=24$)

Baseline characteristics	*Shedders* (n = *6*)	*Nonshedders* (n = *24*)	p-*value*
Mean age (years) ± SD	28.5 ± 2.1	32.5 ± 1.5	0.23
Reproductive history			
Hormonal contraception use, *N* (%)	50	41.7	0.14
Average age at first intercourse ± SD	16.7 ± 0.7	17.3 ± 0.4	0.41
Lifetime number of sexual partners (mean, SD)	4.7 ± 1.3	3.9 ± 0.36	0.41
Number of current sexual partners (median, range)	0.83 ± 0.17	0.67 ± 0.10	0.44
Gravidity (median, range)	2.2 ± 0.6	3.2 ± 1.7	0.19
Parity (median, range)	2.2 ± 0.6	2.8 ± 0.3	0.37
HIV history			
CD4 count/μl, mean ± SD	264.6	400.7	0.40
WHO stage (%)			
1	16.7	25.0	0.45
2	33.3	20.8	
3	16.7	41.7	
4	33.3	12.5	
Duration of HAART use, mean (months) ± SD	17.6 ± 4.0	19.6 ± 4.6	0.85
Number with detectable baseline plasma HIV-1 RNA	2	6	n/a
Baseline plasma HIV-1 RNA viral load, mean copies/ml	12,323 ± 12,309	97.4 ± 65.3	0.04
Detectable baseline genital HIV-1 RNA	5	n/a	n/a
Genital HIV-1 viral load range at baseline	174–7,167	n/a	n/a

and follow-up visits, we had limited statistical power to assess associations with individual predictors. Further studies could examine the possibility that incomplete response to HAART or preclinical treatment failure could result in increased HIV-1 RNA genital shedding for a small population of women.

Baeten *et al.*[4] recently reported a link between higher genital HIV-1 RNA levels and increased risk of HIV transmission, independent of blood plasma HIV-1 concentrations. The group found that genital HIV-1 levels were significantly higher among those who did compared to those who did not transmit HIV-1 (3.89 vs. 3.18 $\log_{10}$ copies/endocervical swab, $p<0.01$). No women in our cohort had HIV-1 genital levels that were as high as the mean levels found in transmitters in that study. Only three women in our study (Subjects #11, 19, and 20) had genital HIV-1 RNA levels that were higher than the mean level found in the nontransmitters in the Baeten study (3.47, 3.86, and 3.60 log cps), indicating the potential to have increased risk of transmission of the HIV-1 virus. However, in two of these cases (#19 and 20), the elevated HIV-1 RNA concentration was noted at baseline, and in fact decreased after biopsy. Although the absolute quantity of HIV-1 in genital secretions may be different in this study than in the Baeten study due to the difference in collection methods, we found that biopsy had no correlation with increased genital levels, and by inference, no increased risk of HIV-1 transmission.

Our study has several strengths. Women were followed prospectively, so it was possible to compare HIV-1 RNA in genital secretions in women before and up to 7 days after cervical biopsy. We were able to control for many of the confounding factors that might impact HIV-1 RNA genital shedding, including STIs, grade of CIN, and hormonal contraception. By excluding all these factors, we were more able to directly evaluate the potential role of cervical biopsy on HIV-1 RNA genital shedding in women on HAART.

Table 2. HIV-1 RNA Measurement and HAART Use Among the Six Women Who Had Detectable Cervical HIV-1 RNA Before and/or After Biopsy

	Subject number					
Clinical characteristics	*4*	*9*	*11*	*17*	*19*	*20*
Baseline cervical HIV-1 RNA (copies/sample)[a]	174	—	564	297	7,167	3,954
Cervical HIV-1 RNA day 2 after biopsy (copies/sample)	—	183	948	1,311	3,267	1,854
Cervical HIV-1 RNA day 7 after biopsy (copies/sample)	—	—	2,958	—	4,653	1,935
Plasma HIV-1 RNA (copies/sample)[a]	—	—	—	68	73,871	—
CD4 count (cells/μl)	15	1,088	88	150	16	231
HAART duration (months)	4.5	5.6	35.3	52.8	28.8	11.9
First or second line therapy[b]	First	First	Second	First	First	First

[a]Lower limit of detection for both plasma HIV-1 RNA secretion levels and cervical HIV-1 RNA secretion levels was 40 copies/sample.
[b]First line antiretroviral therapy: two nucleoside reverse transcriptase inhibitors plus one nonnucleoside reverse transcriptase inhibitor. Second line antiretroviral therapy: two nucleoside reverse transcriptase inhibitors plus one protease inhibitor.
—, undetectable level.

Our study also has several limitations. The relatively small number of women in our sample limited our power, especially for multivariable analyses. Furthermore, we had many exclusion criteria for study participants. Although this decision was made deliberately to minimize confounding, and examine shedding in a specific population, it limits the generalizability of our findings. We examined only women who were on HAART, and so the effect of cervical biopsy on HIV-1 RNA genital shedding levels for women not on HAART remains unknown. An inverse relationship between $CD4^+$ count and genital HIV-1 concentrations has been demonstrated,[22] suggesting that women eligible for HAART would be at highest risk for shedding. Women who have access to cervical cancer screening are more likely to have access to HIV care, and so would presumably be started on HAART if medically indicated. Therefore, although the relationship between biopsy and HIV-1 genital shedding in HAART-naive women is an important unanswered question, understanding the pattern for women on HAART may be more applicable to trends in current HIV treatment programs.

Our study provides the first evidence that cervical biopsy performed in HIV-infected women on HAART does not increase detection or concentration of HIV-1 RNA in genital secretions. This lends support to the use of cervical biopsy in HIV-1-infected women in order to increase the accuracy of the diagnosis prior to treatment for CIN, and suggests that performing a cervical biopsy will not impact the risk for female-to-male HIV-1 transmission. This new information, along with the recent study that cervical procedures did not appear to be associated with HIV-1 acquisition,[4] helps support the safety of using cervical biopsy in an HIV-1-infected population. The next steps are to compare HIV-1 RNA genital shedding after treatment of CIN with LEEP or cryotherapy. Initial work has already shown that cryotherapy does not appear to have an effect on HIV genital shedding.[23] Further evaluation examining HAART's protective effect against HIV-1 RNA genital shedding after LEEP and cryotherapy could influence clinical recommendations on treatment modality for CIN among HIV-1-infected women.

In conclusion, among women stable on HAART for at least 3 months, no increase in HIV-1 RNA concentration in genital secretions was observed after cervical biopsy, supporting the safety of this procedure in similar populations.

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Author Disclosure Statement

No competing financial interests exist.

References

1. Human Papillomavirus and Related Cancers. Summary Report Update. Africa. 2010. Available at www.who.int/hpvcentre/en/. Accessed May 15, 2011.
2. UNAIDS Global report 2010/UNAIDS Report on the global AIDS epidemic 2010. Available at www.unaids.org/globalreport/Global_report.htm. Accessed May 1, 2011.
3. Diouf K, Sawaya GF, Shiboski S, *et al.:* Investigating potential associations between cervical procedures and HIV acquisition. ISRN Obstet Gynecol 2011;2011: 789106.
4. Baeten JM, Kahle E, Lingappa JR, *et al.:* Genital HIV-1 RNA predicts risk of heterosexual HIV-1 transmission. Sci Translation Med 2011;3:77ra29.
5. LeGoff J, Weiss HA, Gresenguet G, *et al.:* Cervicovaginal HIV-1 and herpes simplex virus type 2 shedding during genital ulcer disease episodes. AIDS 2007;21:1569–1578.
6. Hashemi FB, Ghassemi M, Faro S, *et al.:* Induction of human immunodeficiency virus type 1 expression by anaerobes associated with bacterial vaginosis. J Infect Dis 2000;181: 1574–1580.
7. Coleman JS, Hitti J, Bukusi EA, *et al.:* Infectious correlates of HIV-1 shedding in the female upper and lower genital tracts. AIDS 2007;21:755–759.
8. Kilmarx PH, Mock PA, and Levine WC: Effect of Chlamydia trachomatis coinfection on HIV shedding in genital tract secretions. Sex Transm Dis 2001;28:347–348.
9. Ghys PD, Fransen K, Diallo MO, *et al.:* The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Côte d'Ivoire. AIDS 1997;11:F85–F93.
10. Mostad SB and Kreiss JK: Shedding of HIV-1 in the genital tract. AIDS 1996;10:1305–1315.
11. Wright TC Jr, Subbarao S, Ellerbrock TV, *et al.:* Human immunodeficiency virus 1 expression in the female genital tract in association with cervical inflammation and ulceration. Am J Obstet Gynecol 2001;184:279–285.
12. Oster AM, Sullivan PS, and Blair JM: Prevalence of cervical cancer screening of HIV-infected women in the United States. J Acquir Immune Defic Syndr 2009;51:430.
13. Fiore JR, Suligoi B, Saracino A, *et al.:* Correlates of HIV-1 shedding in cervicovaginal secretions and effects of antiretroviral therapies. AIDS 2003;17:2169–2176.
14. Cu-Uvin S, Caliendo AM, Reinert S, *et al.:* Effect of highly active antiretroviral therapy on cervicovaginal HIV-1 RNA. AIDS 2000;14:415–421.
15. Graham SM, Masese L, Gitau R, Jalalian-Lechak Z, *et al.:* Antiretroviral adherence and development of drug resistance are the strongest predictors of genital HIV-1 shedding among women initiating treatment. J Infect Dis 2010;202: 1538–1542.
16. Gitau RW, Grahm SM, Masese LN, *et al.:* Effect of acquisition and treatment of cervical infections on HIV-1 shedding in women on antiretroviral therapy. AIDS 2010;24:2733–2737.
17. Neely MN, Benning L, Xu J, *et al.:* Cervical shedding of HIV-1 RNA among women with low levels of viremia while

receiving highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2007;44:38–42.
18. Spinillo A, Zara F, Gardella B, *et al.:* Cervical intraepithelial neoplasia and cervicovaginal shedding of human immunodeficiency virus. Obstet Gynecol 2006;107:314–320.
19. Tang N, Huang S, Salituro J, *et al.:* A RealTime HIV-1 viral load assay for automated quantitation of HIV-1 RNA in genetically diverse group M subtypes A-H, group O and group N samples. J Virol Methods 2007;146:236–245.
20. Henning TR, Kissinger P, Lacour N, *et al.:* Elevated cervical white blood cell infiltrate is associated with genital HIV detection in a longitudinal cohort of antiretroviral therapy-adherent women. J Infect Dis 2010;202:1543–1552.
21. Balmer DH, Gikundi E, Kanyotu M, *et al.:* The negotiation strategies determining coitus in stable heterosexual relationships. Health Transit Rev 1995;5:85–95.
22. McClelland RS, Beaten JM, Richard BA, *et al.:* A comparison of genital HIV-1 shedding and sexual risk behavior among Kenyan women based on eligibility for initiation of HAART according to WHO guidelines. J Acquir Immune Defic Syndr 2006;41:611–615.
23. Chung MH, McKenzie KP, Richardson BA, *et al.:* Cervical HIV-1 RNA shedding after cryotherapy among HIV-positive women with cervical intraepithelial neoplasia stage 2 or 3. AIDS 2011;25:1915–1919.

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