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Plasma and Cervical Viral Loads among Ugandan and Zimbabwean Women during Acute and Early HIV-1 Infection

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Introduction

Early HIV infection represents a dynamic period during which infection disseminates from local lymph nodes and HIV viremia initially climbs to very high levels followed by a decline to an equilibrium (viral setpoint). The high viremia levels in peripheral blood during early infection appear associated with high levels of HIV transmission [1]. In addition, the level of viral setpoint is an important predictor of subsequent HIV disease progression [2-4].

While this dynamic period has been well-documented in the peripheral blood, little is known about the dynamic of genital viral loads during early infection (first 6 months). Genital viral loads, the biologic mediator between plasma viral loads and HIV transmission, are important to understand particularly during the early infection period. Two recent studies conducted in men [5] and women [6] have documented high genital viral loads during this period. However, it is unclear if and when a setpoint is attained in the genital compartment. It is also unclear whether there are modifiable risk factors that influence genital viral loads during the early infection period.

Few studies have examined factors associated with plasma viral setpoint. A study conducted among Kenyan sex workers [7] found that use of the injectable-progestin contraceptive depot-medroxyprogesterone acetate (DMPA) at the time of HIV infection was associated with a higher plasma viral load setpoint while the presence of genital ulcer disease (GUD)

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during early HIV infection was associated with higher subsequent plasma viral loads. However, these findings have not been corroborated.

HIV-1 genital shedding among women can be affected by both systemic (pregnancy, hormonal contraceptive use, CD4 lymphocyte levels, plasma viral loads, HAART, HIV-1 subtype) [8-15] and local factors (menstruation, genital inflammation, cervical and vaginal infections, abnormal vaginal flora) [6;8-9;13;16-20]. However, factors associated with HIV-1 genital shedding during early HIV-1 infection have not been reported.

We studied the relationship between plasma and genital HIV viral loads among Ugandan and Zimbabwean women during acute and early (first 6 months) HIV infection and examine factors, including hormonal contraceptive use, that may be associated with plasma and genital viral loads during this period and could impact subsequent HIV-1 disease progression or transmission. Data was drawn from a prospective cohort study of contraception and HIV acquisition – the Hormonal Contraception and the Risk of HIV Acquisition (HC-HIV) Study [21] and a subsequent study conducted among the women who became HIV-infected – the Hormonal Contraception and HIV-1 Genital Shedding and Disease Progression among Women with Primary HIV Infection (GS) Study.

Methods

The research was approved by the institutional review boards of the collaborating institutions. All participants provided written informed consent prior to study participation.

Study Population and Procedures

The study population was drawn from women who enrolled in the GS Study during the period from 2001-2007. The 188 GS Study participants were HIV-infected (with known infection dates), ages 18-45 years, were using either no hormonal method, COCs (low-dose pills containing 30 mcg ethinyl estradiol and 150 mcg of levonorgestrel) or DMPA (150 mg depot-medroxyprogesterone acetate administered quarterly). Women were ineligible for enrollment if they had a hysterectomy, a spontaneous or induced abortion within 10 days of enrollment or were using hormonal contraception besides COCs or DMPA.

GS Study procedures were similar to those previously described for the HC-HIV Study [21]. Briefly, when HC-HIV study participants were notified of their HIV-infection, they were informed about the GS Study. Interested women were scheduled as soon as possible for a GS enrollment visit where informed consent procedures were conducted. At enrollment participants were interviewed in the local language to collect sexual behavior, reproductive health and contraceptive history data. We provided contraceptive, HIV risk reduction and condom use counseling and free contraceptives and condoms. Study clinicians conducted a standardized physical (including pelvic) exam and collected specimens for reproductive tract infections, pregnancy testing, Pap smears, lymphocyte phenotyping and plasma and cervical viral loads. Testing for reproductive tract infection and pregnancy was done as previously described [21]. Participants were treated onsite for vaginal infections; women diagnosed with asymptomatic chlamydia, gonorrhea or syphilis were recalled for treatment.

Follow-up visits were conducted at 4, 8 and 12 weeks following enrollment and at 12-week intervals thereafter. Follow-up procedures were similar to those at enrollment and included testing for all STIs (syphilis testing and Pap smears were conducted every 6 months).

Beginning in 2003, women who developed severe symptoms of HIV infection (WHO clinical stage IV or advanced stage III disease), or who had successive CD4 lymphocyte counts of ≤200 cells/mm^3 were offered highly active antiretroviral therapy (HAART) and

trimethoprim-sulfamethoxazole (for prophylaxis against bacterial infections). At each study visit participants were provided with daily multivitamins and iron.

Analysis Population and Variable Definition

The analysis population for the calculation of plasma and cervical viral setpoints included 188 Ugandan and Zimbabwean women contributing 1,042 plasma and 813 cervical specimens within 24 months of HIV-1 infection. The analysis population for comparison of plasma and genital viral loads during early infection included 173 women providing 528 plasma specimens and 159 women providing 471 cervical specimens evaluated within 6 months of HIV-1 infection.

HIV PCR was performed on samples from visits prior to HIV seroconversion to establish infection timing [21]. For women whose seroconversion visit was also their first PCR+ visit, HIV-1 infection dates were estimated as the midpoint between this and the previous visit. Because HIV testing was conducted every 12 weeks in the HC-HIV Study, estimated infection date was usually within a 6-week window of the actual infection date. We defined acute infections as those that were serologically negative but HIV PCR+. We estimated acute infections to occur 15 days prior to the first PCR+ visit.

Contraceptive exposure definition varied by analysis. For analyses associated with viral setpoint, exposed women were those using COCs or DMPA between the two study visits where the HIV infection occurred. For the comparison of plasma and genital viral loads, exposed women were those using COC or DMPA during the 12-week period prior to the specimen collection visit. When women switched from DMPA to the non-hormonal (NH) group, we calculated DMPA exposure as 120 days from the last injection.

Viral RNA load determination

We obtained samples for cervical viral loads by inserting a dacron swab into the endocervix and rotating the swab for 3-5 seconds [22]. The swabs were then stored in 1 ml of RNALater at -70° C. Due to the viscosity of RNALater, virus was pelleted by diluting the 500 μl of RNAlater to a final volume of 8 ml with RPMI. After centrifugation at 23,600 × G for 3 hours at 4° C, the entire viral pellet was resuspended and viral loads estimated according to the ultrasensitive (lower limit of detection =50 copies/ml) procedure of the Roche Amplicor HIV-1 Monitor Test, version 1.5. Details of this methodology are provided in the Supplementary Methods. Plasma viral loads were also performed using the same Roche Amplicor version 1.5 assay as per the manufacturers' protocol. If plasma viral loads were <400 copies/ml, repeat analyses were performed using the ultrasensitive procedure to obtain a sensitivity of 50 copies/ml. Likewise, plasma was diluted 100-fold and the standard Roche Amplicor 1.5 assay was repeated if the initial viral load was >750,000 copies/ml.

HIV-1 DNA sequencing, subtype determination, and prediction of co-receptor usage

To determine HIV-1 subtypes, DNA was extracted from whole blood using the Qiagen DNA extraction kit (Qiagen Inc, Maryland). The *env* gene was PCR amplified in the C2-V3 region using an external-nested PCR amplification with primer pairs ENV B-ED14 (external) and ENV1-ENV2 (nested) [23]. The primer sequences are provided in Supplementary Table 1. PCR products were purified using the Qiagen PCR purification kit then sequenced using the Beckman Coulter CEQ 8000 sequencer using the ENV1 forward primer. Sequences were analyzed and edited as described in the Supplementary Methods. These HIV-1 sequences are available in Genbank (numbers are currently being obtained).

Statistical Methods

We used the Loess procedure to estimate the mean level and timing of plasma and cervical viral setpoints [24]. A marginal model with generalized estimating equation (GEE) approach (to account for repeated measurements on the same individual) was used to determine the plasma viral setpoint among various exposure groups and to model the association between predictors (the difference in viral setpoint between those with and without a specified characteristic) and plasma HIV-1 viral load within the defined time period.

We used Spearman's correlation coefficient to measure correlation between plasma and genital viral loads. We used marginal models using the GEE approach for hypothesis testing of the comparison of genital viral loads levels over time and to evaluate the impact of covariates on cervical HIV-1 RNA levels during early (≤6 months) HIV infection.

Because all Zimbabwean participants with completed subtyping (n=72) are subtype C, we imputed subtypes for the remaining 57 Zimbabwean participants. Additionally, 2 Ugandan participants with subtype C infections were dropped from multivariate modeling due to small group size.

Results

Of the 188 Ugandan and Zimbabwean women contributing data to these analyses, 129 (69%) were Zimbabwean and 59 (31%) were Ugandan.

Participant Characteristics at HIV Seroconversion Visit

At HIV seroconversion the median age was 25 years and median education was 10 years (Table 1). About two-thirds of women used hormonal contraception including DMPA (40%) and COCs (30%). Only 4% of women were currently pregnant while 13% currently breastfed. Few participants reported multiple sex partners (3%), commercial sex (2%), or having a new sex partner (4%). One quarter of women reported consistent condom use during the previous 3 months. The prevalence of STIs was high: 13 women (7%) had a chlamydial infection, 24 women (14%) had gonorrhea, 55 (31%) women had bacterial vaginosis and most (85%) were HSV-2 positive.

At the HIV infection visit NH participants were slightly older and more likely to be pregnant than COC or DMPA users (Table 1). NH participants also had higher levels of sexual risk including more commercial sex and a higher number of partners spending nights away from home but also reported more consistent condom use (49%) than HC users (13%). No important differences were found in STI prevalence between contraceptive groups (Table 1).

All Zimbabwean participants had subtype C HIV infections, while 34 Ugandan participants (63%) had subtype A, 18 (33%) had subtype D and 2 (4%) had subtype C infections. A subset of these sequences is presented in a phylogenetic neighbor joining tree (see supplementary materials).

CXCR4-usage or dual tropism was only predicted in four participants at the time of early infection in this cohort [25-26]. Of these four participants, one was subtype A, one subtype D and two were subtype C (both from Zimbabwe).

Analysis of HIV Viral Setpoint

We estimated the population mean HIV-1 plasma viral setpoint to be 4.20 $\log_{10}$ HIV-1 copies/ml (95% CI 4.04, 4.35) at 121 days (95% CI, 91-137) from the HIV infection date (Figure 1). Mean viral load at setpoint for participants with dual tropic virus (4.34 copies/ml) was similar to that for the entire analysis population. The crude mean and standard error

for HIV plasma viral load (from 121 days to 24 months) was 4.17 $\log_{10}$ HIV-1 copies/ml (SE=0.04). Multivariable analysis was used to assess the effect of a variety of factors on the estimated mean setpoint. In multivariable analysis, contraceptive (including COC and DMPA) use, STI (chlamydia and gonorrhea), and sexual risk behaviors at the time of HIV infection were not significantly associated with the plasma viral setpoint (Table 2). Younger age (18-24 years) was associated with a decrease in mean viral setpoint of -0.30 $\log_{10}$ HIV-1 copies/ml (95% CI -0.58, -0.02) compared with older age and subtype D (compared to subype A) infection was associated with an increase in mean viral setpoint of +0.48 $\log_{10}$ HIV-1 copies/ml (95% CI 0.01, 0.94). Both pregnancy (+0.48 copies/ml) and breastfeeding at the time of infection (+0.54 copies/ml) were also significantly associated with an increase in mean viral setpoint. Following the establishment of the mean plasma viral setpoint, subsequent plasma viral loads increased only slightly (+0.005 $\log_{10}$ HIV-1 copies/ml per month; p=0.24) through 24 months.

We also found a significant difference in the time to mean plasma viral setpoint by HIV-1 subtype. Time to setpoint was fastest for subtype D (100 days; 95% CI 67-109 days) followed by subtype A (139 days; 95% CI 109-157 days) and was slowest for subtype C infections (183 days; 95% CI 152-200 days).

Genital Viral Loads during Early Infection

We observed a direct correlation between HIV-1 cervical and plasma viral RNA levels during early infection (Spearman's r = 0.47 p<.0001) (Figure 1). We found an equilibrium level or 'setpoint' among genital secretions similar to that in the peripheral blood. The mean cervical setpoint was 1.64 $\log_{10}$ HIV-1 copies/swab (95% CI: 1.46-1.82) and occurred at 174 days (95% CI, 145-194) from the estimated infection date. Similar to plasma viral loads, cervical viral loads were higher during acute infection (mean of 3.01 $\log_{10}$ copies/swab) than during periods 1-2, 2-4 and 4-6 months post-infection (means of 2.30, 2.00, and 1.92 $\log_{10}$ HIV-1 copies/swab; p=0.03, p<0.01 and p<0.01, respectively) (Table 3). Cervical specimens taken 1-2 months after HIV-1 infection had higher mean viral loads than specimens taken 2-4 and 4-6 months from time of infection (p<0.01). The comparisons were similar when each country was considered individually. Following the establishment of a setpoint at 174 days post-infection, cervical viral loads did not change significantly (+0.001 $\log_{10}$ HIV-1 copies/swab per month; p=0.85) through 24 months.

In multivariable analysis, having a non-viral STI (chlamydia, gonorrhea or trichomoniasis) (+ 0.29 $\log_{10}$ copies/swab; p=0.03), a partner spending nights away from home (+ 0.22 $\log_{10}$ copies/swab; p<0.01), unprotected sex within 3 days (+ 0.21 $\log_{10}$ copies/swab; p = 0.06) and Zimbabwe-subtype C infection (+ 0.26 $\log_{10}$ copies/swab; p = 0.05) were associated with increased cervical viral loads (Table 4). The effect of subtype D infection on mean cervical viral load (+ 0.30 $\log_{10}$ copies/swab) was of similar magnitude as subtype C infection but was not statistically significant (p=0.09). Greater duration since HIV infection (- 0.11 $\log_{10}$ copies/swab per month; p < 0.01) was associated with decreased cervical viral loads. There was no association between DMPA (+ 0.12 $\log_{10}$ copies/swab; p=0.35) or COC use (+ 0.08 $\log_{10}$ copies/swab; p=0.50) and cervical HIV-1 viral loads. Age, pregnancy, breastfeeding, and genital ulcer disease were also not significantly associated with cervical HIV-1 levels.

We also considered our final multivariate model predicting cervical viral loads adjusted for plasma viral load. Higher plasma viral loads were strongly associated with higher mean cervical loads (+ 0.30 $\log_{10}$ copies/swab; p =<0.001) and time since HIV infection remained strongly associated with decreased mean cervical loads (-0.09 $\log_{10}$ copies/swab per month; p <0.001). Having a partner who spent nights away from home also remained associated with higher cervical loads (+ 0.20 $\log_{10}$ copies/swab; p <0.01). However HIV-1 subtype,

non-viral STIs and having unprotected sex within the last 3 days were no longer significantly associated with mean cervical loads. Instead, breastfeeding (+ 0.25 $\log_{10}$ copies/swab; p = 0.04) and the number of coital acts per month (15-29 acts: +0.17 $\log_{10}$ copies/swab; p = 0.04; ≥30 acts: + 0.35 $\log_{10}$ copies/swab; p = 0.18) were associated with higher cervical viral loads.

Discussion

We found that women in Uganda and Zimbabwe established a plasma viral setpoint of 4.20 $\log_{10}$ HIV-1 copies/ml at 121 days and an analogous cervical viral 'setpoint' of 1.64 $\log_{10}$ HIV-1 copies/swab at 174 days from estimated date of HIV-1 infection suggesting that setpoint is achieved later in the genital compartment. Cervical viral loads were strongly correlated with plasma viral loads during the first 6 months of HIV-1 infection (p<.0001) and were significantly higher (0.7-1.1 $\log_{10}$ copies/ml higher) during acute infection than subsequently during the early infection period.

Our findings concerning the level and timing of the plasma viral setpoint are similar to those reported by other studies. For example, a study of 161 sex workers in Mombasa, Kenya reported a median viral setpoint of 4.46 $\log_{10}$ copies/ml attained at 4 months post-infection [7]. A study of high-risk Kenyan men and women found a virus setpoint of 4.60 $\log_{10}$ copies/ml at 209 days post-infection [27]. Similarly, a study among newly HIV-infected adults in the U.S. estimated the viral setpoint at 4.56 $\log_{10}$ copies/ml at 117 day post-infection [24].

We found that subtype D infection, pregnancy and breastfeeding at the time of HIV infection were associated with a higher plasma viral setpoint while young age was associated with a decreased plasma setpoint. These findings concerning predictors of plasma viral setpoint contrast with a previous study conducted among Kenyan sex workers. In that study, DMPA use was associated with a higher viral setpoint (compared with no use of hormonal contraception) but no association was reported between older age, pregnancy, breastfeeding or subtype D infection and plasma viral setpoint [7]. While no other analyses of predictors of viral setpoint exist, several studies have reported on predictors of HIV-1 disease progression. A Zambian study found an increased risk of disease progression (CD4 < 200 cells/mm^3 or death) among women using hormonal contraception compared with women randomized to copper IUDs [28]. Conversely, a study of postpartum Kenyan women found no differences in change in plasma viral load or CD4 counts among women initiating COCs or DMPA [29]. Additionally, several studies suggest that older age [30] and subtype D HIV-1 infection [31-34] are associated with more rapid HIV-1 disease progression. On the other hand, most studies conclude that pregnancy, while causing transient CD4 decline, is not associated with more rapid disease progression [35-38].

We found a dynamic in the female genital compartment similar to the plasma viral setpoint - high levels of HIV-1 genital viremia during acute infection falling to a steady-state level at about 6 months. Following the establishment of this 'setpoint,' genital viral loads remained constant up to 2 years post-infection. We are not aware of previous reports of a 'setpoint' in the genital compartment. Most previous studies have not had substantial genital viral load data from the acute and early infection periods. However, while it is well-documented that the plasma viral setpoint is predictive of subsequent disease progression [2-4], the utility of a genital 'setpoint' as a predictor of potential infectivity to a sex partner remains to be established.

Our findings corroborate recent reports of high levels of HIV-1 genital shedding early in infection in both women and men with declining levels thereafter [5-6]. Genital and plasma

viral loads have also been strongly correlated in other studies (r = 0.4 to 0.7) [20;39-41]; plasma RNA load is often the factor most strongly associated with genital RNA load in multivariable models [40;42]. However the strong correlation between genital and plasma viral loads has not previously been clearly documented during early infection.

Subtype C infection, non-viral STIs, having a partner who spends nights away from home and recent unprotected sex were associated with higher cervical HIV-1 loads while time since infection was associated with decreased cervical loads. Hormonal contraceptive (COC and DMPA) use was not associated with cervical viral loads during early HIV-1 infection. Our results corroborate the findings of much previous research. For example previous studies have identified non-viral STIs [6;9;40;43], recent unprotected sex and subtype C HIV-1 infection [16] as associated with higher genital viral loads. Our findings that hormonal contraception is not associated with HIV RNA genital shedding also agrees with most (but not all) previous studies suggesting that hormonal contraception appears to be associated with shedding of HIV-infected cells (measured by HIV-1 DNA) but not cell-free virus (measured by HIV-1 RNA) in the female genital tract [8-10;12;15;20]. However, we are unaware of previous research assessing correlates of HIV-1 genital shedding among women during early infection.

Our study has a number of important strengths. The study was prospective with samples for both plasma and cervical viral loads being collected every 12 weeks beginning before HIV infection. We measured HIV infection timing with precision by conducting HIV PCR testing on serial samples that were serologically negative. We accurately measured many variables that were potentially associated with both HIV viral setpoint and genital shedding including hormonal contraceptive use and reproductive tract infections. We also measured viral subtype from women with a variety non-B HIV-1 clades. Finally, we enrolled women seeking family planning services in two sub-Saharan countries. This allows for greater generalizability of study results than a study population drawn from a selected high-risk group (e.g. sex workers).

Our study also had limitations. We used RNAlater for storage media for cervical specimens. This resulted in lower cervical viral load levels than for specimens collected in DMSO (compared at later study visits). We only sequenced the C2-V3 region of env and thus cannot fully explore the issue of recombinant viruses. Also, some women had unprotected sex during the 3 days prior to their study visit and thus measured genital viral loads at these visits could have been a combination of the participant's and her partner's viral load. However, we measured unprotected sex acts in the last 3 days and adjusted for this in our model of cervical viral loads and believe that this improves the accuracy of our estimates of predictors of cervical viral loads (Table 4). Finally, we are unable to address whether a genital viral 'setpoint' is meaningful in terms of long-term transmission risk.

In summary, we found that cervical HIV-1 viral loads were highest during acute infection and then declined up to 6 months post-infection where they appeared to reach a setpoint. Factors associated with a higher plasma viral setpoint included older age, subtype D infection, pregnancy and breastfeeding. Factors associated with higher HIV-1 cervical loads during early infection included non-viral STIs, recent unprotected sex, subtype C infection and shorter duration since infection. Modification of these factors could result in slower disease progression (pregnancy, breastfeeding) or HIV-1 transmission risk (prevention of STI and unprotected sex). However, the prognostic value of a cervical viral setpoint on future transmission risk remains to be established.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

C.S.M. is the study principal investigator and directed the design and analysis of the study and wrote the manuscript draft; K.D., S.B., M.M. and B.V.D.P. planned, supervised, conducted (S.B.) and did quality assurance (B.V.D.P.) for the lab work including managing the lab data in Uganda and Zimbabwe; C.K. conducted the data analysis; A.R. monitored the study sites and performed data management; M.D., J.B. and T.C. are site principal investigators and supervised the study teams in Zimbabwe and Uganda; E.A. is the laboratory co-investigator and designed, tested and supervised the virology assays; R.A.S. is the study co-principal investigator and study clinical consultant; all authors contributed to drafts of the manuscript and approved the final manuscript.

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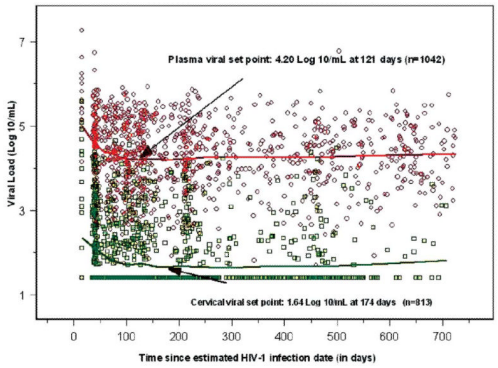

Figure 1.
Plasma and cervical HIV-1 viral load setpoints level and timing

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Table 1

Participant characteristics at the HIV infection visit by contraceptive exposure group[1]

Characteristic	COC (n=52) n (%) or median (Q1-Q3)	DMPA (n=70) n (%) or median Q1-Q3)	Control (n=53) n (%) or median (Q1-Q3)	Total (n=175) n (%) or median (Q1-Q3)	p-value[2]
Sociodemographic					
Study Site: Uganda	15 (29)	21 (30)	18 (34)	54 (31)	0.84
Zimbabwe	37 (71)	49 (70)	35 (66)	121 (69)	
Age at seroconversion	25 (23-29)	25 (23-27)	27 (23-30)	25 (23-29)	0.05
Living with partner[3]	43 (83)	53 (76)	41 (77)	137 (78)	0.64
Number of years in school[3]	10 (8-11)	10 (8-11)	10 (7-11)	10 (8-11)	0.76
Reproductive health and STI history					
Number of lifetime pregnancies[3]	2 (1-3)	2 (2-3)	2 (1-3)	2 (1-3)	0.79
Current Pregnancy	0 (0)	1 (1)	6 (11)	7 (4)	0.01
Current breastfeeding	3 (6)	10 (14)	10 (19)	23 (13)	0.12
STI symptoms[4,5]	13 (25)	24 (34)	18 (34)	55 (31)	0.49
STI history[4,6]	6 (12)	7 (10)	6 (11)	19 (11)	0.96
Sexual risk behavior[4]					
≥2 sex partners	1 (2)	2 (3)	3 (6)	6 (3)	0.67
Commercial sex work	0 (0)	0 (0)	4 (8)	4 (2)	0.01
New sex partner	1 (2)	4 (6)	2 (4)	7 (4)	0.64
Had sex with another man	1 (2)	1 (1)	3 (6)	5 (3)	0.52
Participant behavioral risk[7]	1 (2)	5 (7)	4 (8)	10 (6)	0.40
Participant sexual behavior with all partners					
Number of Coital Acts[8]	12 (8-20)	8 (4-13)	10 (6-16)	10 (6-16)	0.01
Coital frequency[8]:					
0 – 14	32 (62)	53 (76)	37 (70)	122 (70)	0.22
15 – 29	15 (29)	14 (20)	12 (23)	41 (23)	
30+	5 (10)	3 (4)	4 (8)	12 (7)	
Consistent condom use[8]	5 (10)	11 (16)	26 (49)	42 (24)	<.01
Partner nights away from home (last 30 days)	0 (0-10)	0 (0-14)	7 (0-16)	1 (0-15)	0.12

Characteristic	COC (n=52) n (%) or median (Q1-Q3)	DMPA (n=70) n (%) or median Q1-Q3)	Control (n=53) n (%) or median (Q1-Q3)	Total (n=175) n (%) or median (Q1-Q3)	p-value[2]
Partner had sex with another woman[4, 9]	27 (52)	43 (61)	28 (53)	98 (56)	0.50
Primary partner risk[10]	24 (46)	34 (49)	31 (58)	89 (51)	0.42
Clinical/Laboratory Data					
Positive Chlamydia	6 (12)	5 (7)	2 (4)	13 (7)	0.41
Positive Gonorrhea	7 (13)	10 (14)	7 (13)	24 (14)	0.99
Positive Trichomonas	2 (4)	3 (4)	1 (2)	6 (3)	0.88
Positive BV	15 (29)	22 (31)	18 (34)	55 (31)	0.74
Cervical Ectopy	11 (21)	12 (17)	5 (9)	28 (16)	0.33
GUD	2 (4)	0 (0)	0 (0)	2 (1)	0.17
Abnormal vaginal discharge	23 (44)	35 (50)	30 (57)	88 (50)	0.11
Positive HSV-2	43 (83)	58 (83)	48 (91)	149 (85)	0.57
HIV subtype: A	9 (17)	12 (17)	13 (25)	34 (19)	0.85
C	37 (71)	51 (73)	35 (66)	123 (70)	
D	6 (12)	7 (10)	5 (9)	18 (10)	

[1] Based on consistent contraceptive user

[2] Cochran-Mantel-Haenszel test for categorical variables and Fisher's Exact test if a cell is < 5; Kruskal Wallis test for continuous variables

[3] At HC Screening or Baseline Visit

[4] In last 3 months

[5] Includes: abnormal vaginal discharge, genital itching, lower abdominal pain, pain during sex, bleeding between periods

[6] Includes: genital ulcers/sores, genital warts, PID, positive gonorrhea test, positive syphilis test

[7] Includes: having multiple partners or new sex partner or engaged in commercial sex work or had sex with another man in the last 3 months

[8] In a typical month during the last 3 months

[9] Includes: yes and don't know responses

[10] Includes: partner HIV+ or abnormal discharge from penis or weight loss or partner had commercial sex or partner spent nights away from home

Table 2

Analyses of the effect of predictors at the HIV infection visit on HIV-1 plasma viral setpoint (between 121 days – 24 months)

Variable at seroconversion visit	Unadjusted Analysis[7]		Adjusted Analysis[8]	
	Viral set point (95% CI) Log_{10} HIV-1 RNA copies/mL[1] (n=593)	p-value	Viral set point (95% CI) Log_{10} HIV-1 RNA copies/mL[1] (n=565)	p-value
Sociodemographic				
Age < 25 at seroconversion	-0.15 (-0.41, 0.10)	0.25	-0.30 (-0.58, -0.02)	0.04
Country/HIV-1 Subtype				
Uganda: subtype A	Reference		Reference	
subtype C	+1.04 (0.64, 1.45)	<.01	N/A	
Subtype D	+0.44 (0.02, 0.86)	0.04	+0.48 (0.01, 0.94)	0.04
Zimbabwe: subtype C	+0.14 (-0.17, 0.44)	0.38	+0.15 (-0.19, 0.48)	0.38
Consistent Contraceptive Use				
COC	+0.08 (-0.23, 0.39)	0.62	+0.16 (-0.18, 0.51)	0.36
DMPA	+0.12 (-0.18, 0.43)	0.43	+0.10 (-0.22, 0.43)	0.53
Non-hormonal	Reference		Reference	
Reproductive health history[1]				
Current pregnancy	+0.15 (-0.26, 0.55)	0.47	+0.48 (0.04, 0.91)	0.03
Current breastfeeding	+0.31 (0.04, 0.58)	0.02	+0.54 (0.19, 0.90)	<.01
STI symptoms[2,3]	+0.14 (-0.11, 0.39)	0.27	+0.22 (-0.04, 0.48)	0.10
Sexual risk behavior[1,2]				
Participant behavioral risk[4]	+0.18 (-0.41, 0.77)	0.55	+0.19 (-0.44, 0.83)	0.55
Partner had sex with another woman[5]	+0.10 (-0.16, 0.35)	0.46	+0.11 (-0.18, 0.40)	0.45
Primary partner risk[6]	+0.01 (-0.24, 0.26)	0.93	-0.10 (-0.40, 0.19)	0.50
Clinical/Laboratory Data[1]				
Positive Chlamydia	-0.10 (-0.56, 0.35)	0.65	0.00 (-0.48, 0.48)	0.99
Positive Gonorrhea	-0.17 (-0.48, 0.15)	0.30	-0.25 (-0.62, 0.12)	0.19

[1] Increases (+) and decreases (-) in mean viral setpoint are shown as the fraction of log_{10} copies/ml that are attributable to the factor

[2] In the last 3 months

[3] Includes: abnormal vaginal discharge, genital itching, lower abdominal pain, pain during sex, bleeding between periods

[4] Includes: having multiple partners or new sex partner or engaged in commercial sex work or had sex with another man in the last 3 months

[5] Includes: yes and don't know responses

[6] Includes: partner HIV+ or abnormal discharge from penis or weight loss or partner had commercial sex or partner spent nights away from home

[7] The crude mean and standard error for HIV plasma viral load (from 121 days to 24 months) was 4.17 log_{10} HIV-1 copies/ml (SE=0.04).

[8] Two Ugandan women with subtype C were excluded from multivariable model

Table 3

Endocervical HIV-1 viral loads by country during acute and early infection

	Uganda			Zimbabwe			Overall		
	N of Women	**N of Specimens**	**Estimated Mean and SE[1] (Log_{10})**	**N of Women**	**N of Specimens**	**Estimated Meanand SE[1] (Log_{10})**	**N of Women**	**N of Specimens**	**Estimated Meanand SE[1] (Log_{10})**
Acute Infection	48	10	2.83 (0.46)	111	11	3.09 (0.43)	159	21	3.01 (0.31)
1 – 2 months		37	2.23 (0.15)		92	2.32 (0.09)		129	2.30 (0.08)
>2 – 4 months		83	1.88 (0.10)		110	2.06 (0.09)		193	2.00 (0.07)
>4 – 6 months		47	1.85 (0.09)		81	1.95 (0.08)		128	1.92 (0.06)
	P-value[1]								
Acute vs. 1 - 2 months	0.21			0.08			0.03		
Acute vs. >2 - 4 months	0.04			0.02			<.01		
Acute vs. >4 - 6 months	0.03			0.01			<.01		
1 – 2 vs. >2 - 4 months	0.02			0.02			<.01		
>2 – 4 vs. >4 - 6 months	0.68			0.28			0.24		
1 - 2 vs. >4 – 6 months	0.01			<.01			<.01		

[1]Estimated mean and standard error obtained from marginal models using the GEE approach

Table 4

Analyses of the predictor effects of HIV-1 endocervical viral load during early HIV-1 infection (data up to 6 months)

Variable	Unadjusted Analysis[1]		Adjusted Analysis[2]	
	Log_{10} ERNA copies/mL (95% CI) (n=442)[3]	p-value	Log_{10} ERNA copies/mL (95% CI) (n=420)[3]	p-value
Time-invariant variables				
Age < 25 at seroconversion	-0.05 (-0.27, 0.18)	0.69	-0.05 (-0.25, 0.16)	0.66
Country/ HIV-1 Subtype				
Uganda: subtype A	Reference		Reference	
subtype C	+0.24 (-0.81, 1.29)	0.65	N/A	N/A
subtype D	+0.28 (-0.11, 0.67)	0.16	+0.30 (-0.04, 0.64)	0.09
Zimbabwe: subtype C	+0.22 (-0.07, 0.50)	0.13	+0.26 (-0.01, 0.51)	0.05
Time-varying Contraceptive Use at a particular visit				
Consistent COC	+0.12 (-0.13, 0.37)	0.35	+0.08 (-0.15, 0.31)	0.50
Consistent DMPA	+0.12 (-0.11, 0.36)	0.30	+0.12 (-0.13, 0.36)	0.35
Consistent Non-hormonal	Reference		Reference	
Reproductive health and STI history				
Current pregnancy	+0.01 (-0.71, 0.73)	0.98	-0.04 (-0.51, 0.43)	0.85
Current breastfeeding	+0.29 (-0.17, 0.74)	0.22	+0.25 (-0.05, 0.55)	0.10
STI symptoms[4,5]	+0.15 (-0.04, 0.33)	0.12	+0.12 (-0.06, 0.29)	0.18
Sexual risk behavior [4]				
Participant behavioral risk[6]	+0.12 (-0.34, 0.58)	0.60	+0.22 (-0.19, 0.63)	0.28
Coital frequency:[7]				
0 – 14	Reference		Reference	
15 – 29	+0.10 (-0.11, 0.32)	0.35	+0.15 (-0.05, 0.34)	0.14
30+	-0.07 (-0.36, 0.23)	0.66	+0.19 (-0.23, 0.60)	0.38
Unprotected sex act in last 3 days[8]	+0.23 (0.01, 0.45)	0.04	+0.21 (-0.01, 0.44)	0.06
Partner had 1+ nights away from home (last 30 days)	+0.22 (0.06, 0.37)	0.01	+0.22 (0.07, 0.36)	<0.01
Clinical/laboratory Data				
Non-viral STIs[9]	+0.33 (0.07, 0.58)	0.01	+0.29 (-0.02, 0.56)	0.03
GUD	-0.11 (-0.61, 0.38)	0.66	-0.37 (-0.93, 0.19)	0.19
Time variable				
Time since estimated infection date (in month)	-0.14 (-0.19, -0.09)	<0.01	-0.11 (-0.16, -0.06)	<0.01

[1] The crude mean and standard error for HIVendocervical viral load (data up to 6 months) was 2.10 $\log_{10}$ HIV-1 copies/ml (SE=0.04).

[2] Two Ugandan women with subtype C were excluded from multivariable model

[3] Increases (+) and decreases (-) in mean viral setpoint are shown as the fraction of $\log_{10}$ copies/ml that are attributable to the factor

[4] In the last 3 months

[5] Includes: abnormal vaginal discharge, genital itching, lower abdominal pain, pain during sex, bleeding between periods

[6] Includes: having multiple partners or new sex partner or engaged in commercial sex work or had sex with another man in the last 3 months

[7] In a typical month during the last 3 months

[8] Includes: had vaginal intercourse in last 3 days without condom or sperm detected from lab test

[9] Includes: chlamydia, gonorrhea and trichomonas