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Editor's Summary

Elucidating the Insidious Transmission of a Deadly Pathogen

The deadly HIV-1 retrovirus that causes AIDS has been a scourge of humanity for nearly 30 years. Although combination therapy with antiretroviral drugs has proved successful, the complex drug regimen and great cost have prevented their widespread use in the developing world where they are most needed. The goal of developing a vaccine that would protect individuals from becoming infected with HIV-1 has remained elusive. Given that 90% of all HIV infections worldwide are due to sexual transmission, there has been much interest in developing new strategies that could block HIV infection through the genital mucosa. However, the mechanisms underlying mucosal transmission of HIV are still poorly understood. Higher amounts of HIV-1 in genital secretions are thought to reflect a greater chance of sexual transmission, but testing this correlation is a difficult undertaking. Baeten and colleagues have taken on this challenge with their prospective study in Africa of 2521 heterosexual serodiscordant couples (one partner is HIV-infected and the other partner is not). These investigators evaluated the relationship between the quantity of HIV-1 RNA in the genital secretions of the infected partner and the risk of HIV-1 transmission to the uninfected partner in each couple. They tested the amount of HIV-1 RNA in endocervical swabs from 1805 HIV-1 infected women including 46 women known to have transmitted the virus to their male partners. They also tested the amount of HIV-1 RNA in semen from 716 men, including 32 who had transmitted HIV-1 to their female partners. The authors demonstrate that higher concentrations of HIV-1 RNA in genital secretions are associated with a greater risk of heterosexual transmission of HIV-1, and that these concentrations provide a new biomarker for predicting the infectiousness of HIV-1 infected individuals. The authors propose that HIV-1 RNA concentrations in genital secretions could also be used as a biomarker to monitor the efficacy of new microbicides and other interventions designed to block mucosal transmission of the virus.

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HIV

Genital HIV-1 RNA Predicts Risk of Heterosexual HIV-1 Transmission

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High plasma HIV-1 RNA concentrations are associated with an increased risk of HIV-1 transmission. Although plasma and genital HIV-1 RNA concentrations are correlated, no study has evaluated the relationship between genital HIV-1 RNA and the risk of heterosexual HIV-1 transmission. In a prospective study of 2521 African HIV-1 serodiscordant couples, we assessed genital HIV-1 RNA quantity and HIV-1 transmission risk. HIV-1 transmission linkage was established within the partnership by viral sequence analysis. We tested endocervical samples from 1805 women, including 46 who transmitted HIV-1 to their partner, and semen samples from 716 men, including 32 who transmitted HIV-1 to their partner. There was a correlation between genital and plasma HIV-1 RNA concentrations: For endocervical swabs, Spearman's rank correlation coefficient ρ was 0.56, and for semen, ρ was 0.55. Each 1.0 $\log_{10}$ increase in genital HIV-1 RNA was associated with a 2.20-fold (for endocervical swabs: 95% confidence interval, 1.60 to 3.04) and a 1.79-fold (for semen: 95% confidence interval, 1.30 to 2.47) increased risk of HIV-1 transmission. Genital HIV-1 RNA independently predicted HIV-1 transmission risk after adjusting for plasma HIV-1 quantity (hazard ratio, 1.67 for endocervical swabs and 1.68 for semen). Seven female-to-male and four male-to-female HIV-1 transmissions (incidence <1% per year) occurred from persons with undetectable genital HIV-1 RNA, but in all 11 cases, plasma HIV-1 RNA was detected. Thus, higher genital HIV-1 RNA concentrations are associated with greater risk of heterosexual HIV-1 transmission, and this effect was independent of plasma HIV-1 concentrations. These data suggest that HIV-1 RNA in genital secretions could be used as a marker of HIV-1 sexual transmission risk.

INTRODUCTION

Many studies have measured HIV-1 concentrations in semen, cervicovaginal, and anorectal secretions to assess the infectiousness of HIV-1 transmitted sexually (*1*–*3*). Higher genital HIV-1 concentrations are thought to reflect greater HIV-1 infectivity. First, plasma HIV-1 levels predict sexual and perinatal HIV-1 transmission risk (*1*, *4*). Second, higher cervicovaginal HIV-1 concentrations among HIV-1–infected pregnant women have been associated with increased risk of perinatal HIV-1 transmission (*5*, *6*). Third, factors that heighten sexual HIV-1 transmission risk in epidemiological studies, such as genital tract infections resulting in inflammation of the genital mucosa, increase genital HIV-1 (*7*–*13*), and factors that decrease HIV-1 transmission risk, such as antiretroviral therapy (ART) (*14*), decrease genital HIV-1 (*15*). However, although plasma and genital HIV-1 concentrations are correlated, higher variability in mucosal versus blood plasma HIV-1 and discordance between mucosal and plasma HIV-1 in some individuals have raised questions regarding whether genital HIV-1 concentrations can predict the risk of HIV-1 sexual transmission (*1*, *2*, *16*–*18*).

To date, no prospective study has assessed whether genital HIV-1 concentrations correlate with HIV-1 sexual transmission risk. Ninety percent of new HIV-1 infections worldwide are transmitted sexually, and a greater understanding of the biological mechanisms underlying HIV-1 infectiousness is needed. In a prospective cohort of heterosexual African HIV-1 serodiscordant couples, we evaluated the relationship between genital HIV-1 quantity in the HIV-1–infected partner and HIV-1 transmission risk.

RESULTS

Of 3408 HIV-1 serodiscordant couples enrolled in the trial, 2521 (74.0%), including 1805 of 2299 couples with HIV-1 seropositive women (78.5%) and 716 of 1109 couples with HIV-1 seropositive men (64.6%), provided genital samples for HIV-1 RNA quantification and were included in this analysis. The median age was 32 years [interquartile range (IQR), 27 to 38] for HIV-1–infected partners and 34 years (IQR, 28 to 41) for HIV-1–uninfected partners (Table 1). Most couples were married and cohabiting. The median monthly frequency of sex was four times (IQR, 2 to 8), and 28.6% of couples reported unprotected sex during the month before enrollment. Among HIV-1–infected participants, the median $CD4^+$ T cell count was 469 cells/mm^3 (IQR, 350 to 638) and the median plasma HIV-1 RNA concentration was 4.0 $\log_{10}$ copies/ml (IQR, 3.3 to 4.6). There was no statistically significant difference in plasma HIV-1 RNA concentrations

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or $CD4^+$ T cell counts for those who provided versus those who did not provide a genital sample.

Follow-up and HIV-1 seroincidence

During 3509 person-years of follow-up for assessment of HIV-1 seroincidence among the 2521 HIV-1 seronegative partners included in this analysis, 113 partners (73 men and 40 women) seroconverted to HIV-1 (incidence, 3.2 per 100 person-years). Median follow-up was 18 months (IQR, 12 to 24 months). Of the 113 incident HIV-1 infections, 78 (69.0%), including 46 among men (63.0%) and 32 among women (80.0%), were determined by viral sequencing to be linked within the partnership; these frequencies are similar to the study population as a whole (68.9% linked in the overall cohort) (*19*).

Detection and quantity of genital HIV-1 RNA

HIV-1 RNA was detected in 59.9% of endocervical swab samples and 56.5% of semen samples (Table 2); median HIV-1 concentrations were 3.20 $\log_{10}$ copies/swab for endocervical samples and 2.57 $\log_{10}$ copies/ml in semen samples. Genital HIV-1 concentrations were significantly lower among those randomized to receive the drug acyclovir [which suppresses herpes simplex virus type 2 (HSV-2)] versus placebo: median, 2.98 versus 3.29 $\log_{10}$ copies/swab for endocervical swabs ($P < 0.001$) and 2.38 versus 2.76 $\log_{10}$ copies/ml for semen ($P = 0.008$).

Genital HIV-1 RNA concentrations were correlated with plasma HIV-1 levels measured at the closest visit. For 99.6% of endocervical and 63.7% of semen samples, a concurrent plasma sample was collected for HIV-1 RNA quantification; for most of the remainder, a plasma sample for HIV-1 RNA quantification was available within 6 months of collection of the genital sample. Spearman's rank correlation coefficient (ρ) was 0.56 ($P < 0.001$) among women and 0.55 ($P < 0.001$) among men; the correlation was the same (ρ = 0.55, $P < 0.001$) when restricted to those semen samples that had concurrent plasma HIV-1 RNA results. By linear regression, each 1 $\log_{10}$ copies/ml increase in plasma HIV-1 RNA was associated with a 0.52 $\log_{10}$ copies/swab increase in endocervical HIV-1 RNA [95% confidence interval (CI), 0.48 to 0.56; $P < 0.001$] and a 0.46 $\log_{10}$ copies/ml increase in semen HIV-1 RNA (95% CI, 0.40 to 0.52; $P < 0.001$).

Table 1. Enrollment characteristics.

	Median (IQR) or number (%)			
	Couples with HIV-1–infected women (*n* = 1805)		Couples with HIV-1–infected men (*n* = 716)	
	HIV-1–infected female	HIV-1–susceptible male	HIV-1–infected male	HIV-1–susceptible female
*Couple characteristics**				
East Africa (versus southern Africa)	1161 (64.3%)		538 (75.1%)	
Married	1336 (74.0%)		589 (82.3%)	
Living together	1610 (89.2%)		681 (95.1%)	
Duration of partnership (years)	5 (2–9)		6 (3–12)	
Number of children	1 (0–2)		2 (1–3)	
Number of sex acts (previous month)	4 (2–8)		4 (2–8)	
Any unprotected sex acts (previous month)	524 (29.0%)		197 (27.5%)	
Demographic characteristics				
Age (years)	30 (26–35)	35 (30–43)	37 (32–45)	30 (25–37)
Education (years)	8 (6–10)	9 (7–12)	8 (6–11)	8 (6–10)
Any monthly income	431 (23.9%)	1064 (59.0%)	434 (60.6%)	185 (25.8%)
Clinical characteristics				
$CD4^+$ T cell count (cells/mm^3) (HIV-1–infected only)	483 (355–667)	—	437 (343–571)	—
HIV-1 plasma viral load ($\log_{10}$ copies/ml) (HIV-1–infected only)	3.9 (3.2–4.5)	—	4.3 (3.6–4.9)	—
N. gonorrhoeae	29 (1.7%)	8 (0.5%)	5 (0.7%)	8 (1.3%)
C. trachomatis	40 (2.4%)	44 (2.5%)	4 (0.6%)	8 (1.3%)
T. vaginalis	266 (16.0%)	117 (6.6%)	23 (3.3%)	69 (11.1%)
Genital ulcer disease (on examination)	58 (3.2%)	26 (1.4%)	15 (2.1%)	7 (1.0%)
HSV-2 seropositive	1805 (100%)	1082 (59.9%)	716 (100%)	616 (86.0%)
Circumcised (men only)	—	967 (53.6%)	222 (31.0%)	—
Randomized to acyclovir versus placebo (HIV-1–infected only)	897 (49.7%)	—	365 (51.0%)	—

*Couple characteristics were from data collected from the HIV-1–uninfected partner.

For the 46 genetically linked female-to-male HIV-1 transmission events, the median time from endocervical swab collection to the visit at which HIV-1 seroconversion was detected was 5.7 (IQR, 0 to 8.9) months, with 11 (23.9%) samples collected at the same study visit as seroconversion was detected and an additional 20 (43.5%) samples collected within 3 months of the seroconversion visit. Twenty (43.5%) seroconversions occurred after collection of the swab sample. For the 32 male-to-female–linked HIV-1 transmission events, the median time from semen sample collection to HIV-1 seroconversion was 3.0 (IQR, 0 to 6.1) months, with 4 (12.5%) samples collected at the same study visit as seroconversion and an additional 14 (43.8%) samples collected within 3 months of the seroconversion visit. Thirteen (40.6%) seroconversions occurred after collection of the semen sample.

Genital HIV-1 concentrations and HIV-1 transmission risk

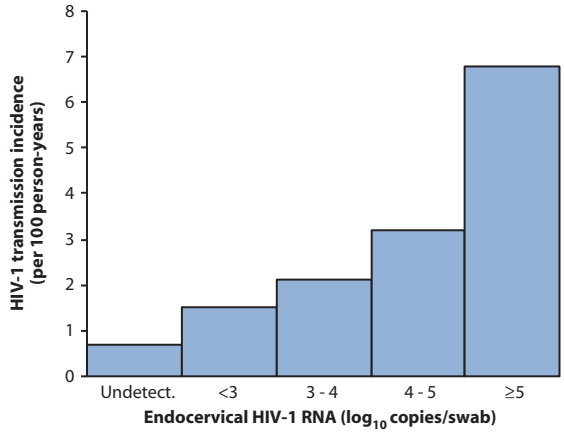
Genital HIV-1 levels were significantly higher among those who did versus those who did not transmit HIV-1: median, 3.89 versus 3.18 $\log_{10}$ copies/swab for endocervical swabs ($P < 0.001$) and 3.44 versus 2.54 $\log_{10}$ copies/ml for semen ($P < 0.001$). A strong stepwise relationship between genital HIV-1 quantity and HIV-1 transmission incidence was observed (Fig. 1); a similar stepwise effect was seen for the relationship between plasma HIV-1 RNA and HIV-1 transmission incidence. In a Cox proportional hazards model, each 1.0 $\log_{10}$ increase in genital HIV-1 RNA was associated with an about twofold greater risk of HIV-1 transmission (Table 3). The hazard ratio (HR) was 2.20 ($P < 0.001$) per $\log_{10}$ copies/swab increase in endocervical HIV-1 RNA and risk of female-to-male HIV-1 transmission, and the HR was 1.79 ($P < 0.001$) per $\log_{10}$ copies/ml increase in semen HIV-1 RNA and risk of male-to-female HIV-1 transmission. This effect of genital HIV-1 RNA concentration remained statistically significant after adjustment for plasma HIV-1 RNA levels and for demographic and clinical characteristics in multivariate analysis. In the final multivariate models, each 1.0 $\log_{10}$ increase in genital HIV-1 RNA increased the risk of female-to-male HIV-1 transmission by 1.67-fold ($P = 0.02$) and the risk of male-to-female HIV-1 transmission by 1.68-fold ($P = 0.02$). Higher plasma HIV-1 RNA concentrations were associated with increased HIV-1 transmission risk, although only the effect on female-to-male transmission was statistically significant (HR, 2.16 per $\log_{10}$ copies/ml increase; $P = 0.001$), whereas the male-to-female HIV-1 transmission effect was not statistically significant (HR, 1.38 per $\log_{10}$ copies/ml increase; $P = 0.2$) in multivariate analysis. Thus, plasma and genital HIV-1 RNA concentrations independently predicted female-to-male HIV-1 transmission risk, but plasma HIV-1 RNA was not significantly associated with male-to-female transmission risk after adjustment for seminal HIV-1 RNA quantity.

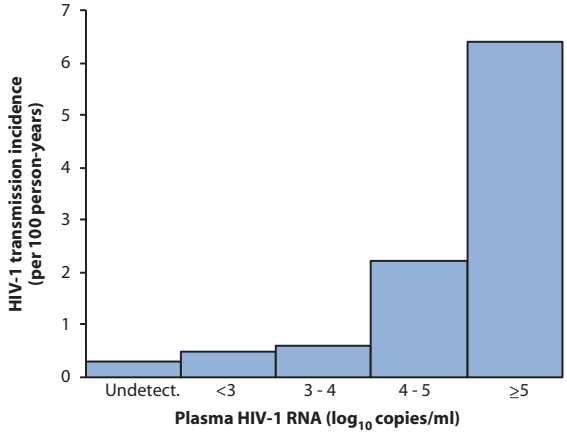
We performed two sensitivity analyses to assess the contribution of timing of genital sample collection to our findings. First, we considered only follow-up time after collection of genital specimens for HIV-1 RNA quantification because genital samples were not collected at study enrollment. We obtained data that were similar to the overall results: multivariate HR 2.38 (95% CI, 1.13 to 4.78) per $\log_{10}$ copies/swab increase in endocervical HIV-1 RNA and risk of

Table 2. Genital and plasma HIV-1 RNA concentrations.

Endocervical swabs (n = 1805)		
HIV-1 RNA detected, n/total (%)	1081/1805	(59.9%)
HIV-1 RNA quantity, $\log_{10}$ copies/swab, median (IQR)	3.20	(2.08–3.87)
HIV-1 RNA quantity, $\log_{10}$ copies/swab, median (IQR), among samples with detectable HIV-1 RNA (n = 1081)	3.74	(3.33–4.24)
Closest plasma HIV-1 RNA sample collected, compared with genital sample collection, n/total (%)		
Same visit as genital sample	1797/1805	(99.6%)
At different visit from genital sample but within 6 months	8/1805	(0.4%)
Correlation of genital and plasma HIV-1 RNA quantities, Spearman's coefficient ρ	0.56 ($P < 0.001$)	
For female-to-male HIV-1 transmissions (n = 46)		
Endocervical swab sample collected at same visit that HIV-1 seroconversion was detected	11/46	(23.9%)
Endocervical swab sample collected at a non-seroconversion visit within 3 months of HIV-1 seroconversion	20/46	(43.5%)
Semen (n = 716)		
HIV-1 RNA detected, n/total (%)	404/716	(56.5%)
HIV-1 RNA quantity, $\log_{10}$ copies/ml, median (IQR)	2.57	(2.08–3.60)
HIV-1 RNA quantity, $\log_{10}$ copies/ml, median (IQR), among samples with detectable HIV-1 RNA (n = 404)	3.44	(2.92–4.12)
Closest plasma HIV-1 RNA sample collected, compared with genital sample collection, n/total (%)		
Same visit as genital sample	456/716	(63.7%)
At different visit than genital sample but within 6 months	251/716	(35.1%)
Correlation of genital and plasma HIV-1 RNA quantities, Spearman's coefficient ρ	0.55 ($P < 0.001$)	
For male-to-female HIV-1 transmissions (n = 32)		
Semen sample collected at same visit when HIV-1 seroconversion was detected	4/32	(12.5%)
Semen sample collected at a non-seroconversion visit within 3 months of HIV-1 seroconversion	14/32	(43.8%)

A Female-to-male HIV-1 transmission

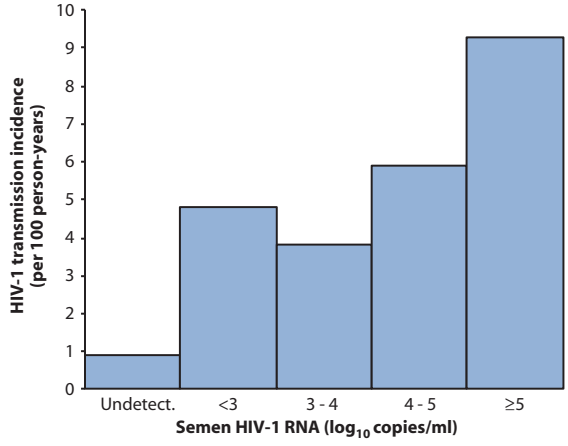


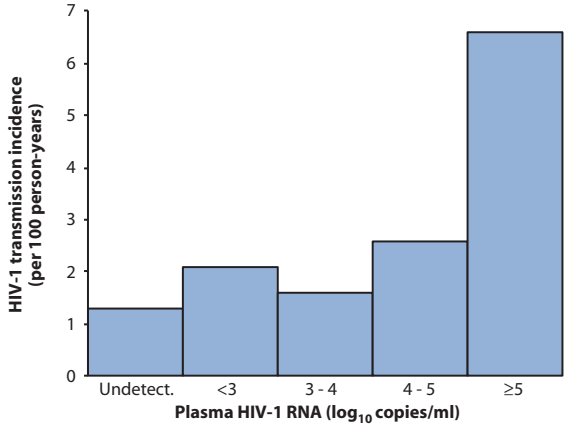


	Log10 endocervical HIV-1 concentration (copies/swab)				
	Undetectable	<3	3-4	4-5	≥5
# Genetically linked HIV-1 seroconversion events	7	2	18	14	5
# Non-transmitters	717	94	581	313	54
Person-years	1093.0	143.6	905.1	475.6	81.0
HIV-1 seroincidence	0.6	1.4	2.0	2.9	6.2
95% confidence interval	0.3-1.3	0.2-4.9	1.2-3.1	1.6-4.9	2.0-13.8

	Log10 plasma HIV-1 concentration (copies/ml)				
	Undetectable	<3	3-4	4-5	≥5
# Genetically linked HIV-1 seroconversion events	1	1	5	20	19
# Non-transmitters	233	147	584	587	191
Person-years	335.4	219.7	898.8	918.2	297.0
HIV-1 seroincidence	0.3	0.5	0.6	2.2	6.4
95% confidence interval	0.01-1.7	0.01-2.5	0.2-1.3	1.3-3.3	3.9-9.8

B Male-to-female HIV-1 transmission





	Log10 semen HIV-1 concentration (copies/ml)				
	Undetectable	<3	3-4	4-5	≥5
# Genetically linked HIV-1 seroconversion events	4	8	9	7	4
# Non-transmitters	307	113	158	79	26
Person-years	488.2	180.2	253.3	130.6	47.4
HIV-1 seroincidence	0.8	4.4	3.6	5.4	8.4
95% confidence interval	0.2-2.1	1.9-8.6	1.6-6.6	2.2-10.7	2.4-20.2

	Log10 plasma HIV-1 concentration (copies/ml)				
	Undetectable	<3	3-4	4-5	≥5
# Genetically linked HIV-1 seroconversion events	1	1	5	12	13
# Non-transmitters	49	30	194	287	117
Person-years	75.2	48.5	305.7	464.5	197.1
HIV-1 seroincidence	1.3	2.1	1.6	2.6	6.6
95% confidence interval	0.03-7.2	0.05-11.0	0.5-3.8	1.3-4.5	3.6-11.0

Fig. 1. Stepwise association between genital and plasma HIV-1 RNA quantity and HIV-1 transmission risk. (**A** and **B**) HIV-1 transmission incidence detailed within categories of HIV-1 RNA quantity for (A) female-to-male and (B) male-to-female HIV-1 transmission. HIV-1 incidence for each HIV-1 RNA quantity category (undetectable, <3, 3 to 4, 4 to 5, and ≥5 $\log_{10}$) is presented for both genital HIV-1 RNA and plasma HIV-1 RNA. A stepwise relationship between HIV-1 RNA quantity and HIV-1 transmission incidence is seen for both genital and plasma HIV-1. The lower limit of quantification was 240 copies/ml for blood and seminal fluid and 240 copies/swab for endocervical samples.

female-to-male HIV-1 transmission, and multivariate HR 2.89 (95% CI, 1.03 to 8.11) per $\log_{10}$ copies/ml increase in semen HIV-1 RNA and risk of male-to-female HIV-1 transmission. Second, we analyzed only those transmitting couples who had a genital sample collected within 3 months of HIV-1 seroconversion. Again, the data were similar to the overall results: multivariate HR 1.88 (95% CI, 1.11 to 3.19) per $\log_{10}$ copies/swab increase in endocervical HIV-1 RNA and risk of female-to-male HIV-1 transmission, and multivariate HR 1.81 (95% CI, 1.04 to 3.14) per $\log_{10}$ copies/ml increase in semen HIV-1 RNA and risk of male-to-female HIV-1 transmission.

Seven of 46 (15.2%) female-to-male HIV-1 transmissions occurred from women with undetectable endocervical HIV-1 RNA concentrations. HIV-1 incidence among the 724 couples in which the women had undetectable HIV-1 RNA concentrations was 0.6 per 100 person-years (95% CI, 0.3 to 1.3). Four of 32 (12.5%) male-to-female HIV-1 transmissions occurred from men with undetectable semen HIV-1 RNA concentrations; HIV-1 transmission from the 311 men who had undetectable semen HIV-1 RNA concentrations was 0.8 per 100 person-years (95% CI, 0.2 to 2.1). For these 11 transmissions, the median time between collection of the genital sample and HIV-1 seroconversion was 4.6 months (range, 0 to 16.3) and all had detectable plasma HIV-1 RNA at the visit closest to collection of the genital sample (median, 4.4 $\log_{10}$ copies/ml; range, 2.4 to 5.9).

DISCUSSION

Our data provide empirical evidence that differences in genital tract concentrations of HIV-1 RNA influence the transmission risk of HIV-1 infection, and we found that this relationship was independent of plasma HIV-1 concentrations. Our large sample size of heterosexual African HIV-1 serodiscordant couples and prospective follow-up with collection of genital samples before HIV-1 transmission permitted analyses demonstrating that the concentration of HIV-1 RNA in endocervical and seminal samples from HIV-1–infected individuals strongly correlated with risk of HIV-1 transmission to their HIV-1–susceptible sexual partners. Genomic analysis of HIV-1 isolates to confirm HIV-1 transmission within the study partnerships further strengthens our findings. These data support the concentration of HIV-1 RNA in genital secretions as a marker of HIV-1 sexual transmission risk.

The first studies of genital HIV-1 using viral culture provided qualitative evidence for infectious virus in genital secretions as a mechanism for HIV-1 transmission (*20*, *21*). Subsequent studies have used nucleic acid amplification to quantify genital HIV-1, with results suggesting that higher genital HIV-1 levels are likely to be a measure of increased HIV-1 infectiousness (*1*). Higher plasma HIV-1 levels, genital tract infections, and advanced HIV-1 disease have been associated

Table 3. Genital HIV-1 RNA predicts HIV-1 transmission risk independent of plasma HIV-1 RNA.

	HR for HIV-1 transmission*	95% CI	*P*
Female-to-male HIV-1 transmission			
Univariate models[†]			
Endocervical HIV-1 RNA (per 1 $\log_{10}$ copies/swab increase)	2.20	(1.60–3.04)	<0.001
Plasma HIV-1 RNA (per 1 $\log_{10}$ copies/ml increase)	2.59	(1.79–3.74)	<0.001
Bivariate model[‡]			
Endocervical HIV-1 RNA (per 1 $\log_{10}$ copies/swab increase)	1.56	(1.08–2.27)	0.02
Plasma HIV-1 RNA (per 1 $\log_{10}$ copies/ml increase)	2.00	(1.32–3.05)	0.001
Multivariate model[§]			
Endocervical HIV-1 RNA (per 1 $\log_{10}$ copies/swab increase)	1.67	(1.10–2.53)	0.02
Plasma HIV-1 RNA (per 1 $\log_{10}$ copies/ml increase)	2.16	(1.36–3.45)	0.001
Male-to-female HIV-1 transmission			
Univariate models[†]			
Semen HIV-1 RNA (per 1 $\log_{10}$ copies/ml increase)	1.79	(1.30–2.47)	<0.001
Plasma HIV-1 RNA (per 1 $\log_{10}$ copies/ml increase)	1.89	(1.20–2.94)	0.006
Bivariate model[‡]			
Semen HIV-1 RNA (per 1 $\log_{10}$ copies/ml increase)	1.61	(1.13–2.29)	0.009
Plasma HIV-1 RNA (per 1 $\log_{10}$ copies/ml increase)	1.52	(0.94–2.45)	0.09
Multivariate model[§]			
Semen HIV-1 RNA (per 1 $\log_{10}$ copies/ml increase)	1.68	(1.08–2.62)	0.02
Plasma HIV-1 RNA (per 1 $\log_{10}$ copies/ml increase)	1.38	(0.81–2.35)	0.2

*All models stratified by HIV-1–infected partner randomization arm (acyclovir versus placebo) and study site, as detailed in Materials and Methods. [†]Univariate models separately assessed genital and plasma HIV-1 RNA concentrations. [‡]Bivariate models include both genital and plasma HIV-1 RNA concentrations (the latter as a time-dependent variable, measured at enrollment; months 3, 6, 9, and 12 after study entry; and at study exit). [§]Multivariate models include genital HIV-1 RNA concentration, plasma HIV-1 RNA concentration (time-dependent), unprotected sex (any versus none, time-dependent), sexually transmitted infection in HIV-1–infected partner (any versus none, at baseline), sexually transmitted infection in HIV-1–uninfected partner (any versus none, at baseline), $CD4^+$ T cell count of the HIV-1–infected partner (time-dependent), HSV-2 serostatus of HIV-1–uninfected partner (at baseline), circumcision status of male partner (at baseline), and age of HIV-1–uninfected partner (at baseline).

with increased genital HIV-1 levels (*2*). In prospective interventional studies with pre- and posttreatment genital tract samples, cure of sexually transmitted infections and initiation of ART significantly reduced genital HIV-1 RNA concentrations (*7*–*9*, *15*, *22*). However, to demonstrate that genital HIV-1 levels predict risk of HIV-1 sexual transmission required longitudinal studies of HIV-1–infected persons and their initially uninfected partners. The establishment of such cohorts has been logistically challenging (*1*). Only one previous case-control study, among men who have sex with men, assessed the relationship between genital HIV-1 RNA concentrations and risk of HIV-1 sexual transmission. This study reported that plasma and seminal fluid HIV-1 RNA concentrations in 15 transmitting partners were significantly higher than in 32 nontransmitting partners (*23*).

We found a stepwise association between genital HIV-1 levels and HIV-1 incidence, with an about twofold increased risk for each one $\log_{10}$ increase in genital HIV-1. This was comparable to the association between endocervical HIV-1 RNA and female-to-male HIV-1 transmission, and seminal HIV-1 RNA and male-to-female transmission. We also found that plasma HIV-1 RNA quantity predicted HIV-1 transmission risk in a similar stepwise manner. This linear risk relationship between $\log_{10}$ HIV-1 RNA concentrations and HIV-1 outcomes has been previously reported for systemic HIV-1 concentrations and both sexual and perinatal HIV-1 transmission (*4*, *24*). The Ugandan study first demonstrated that higher blood HIV-1 concentrations resulted in increased heterosexual infectiousness (*4*) and that plasma HIV-1 levels are a predictor of the risk of HIV-1 clinical progression to AIDS. The consistency of this relationship raises the question of whether the $\log_{10}$ quantity is a fundamental pathogenic property of the virus, although discerning the precise biological mechanism is not possible with the samples we tested in this study.

We observed a small number of HIV-1 transmission events (annual incidence <1%) among couples in which the HIV-1–infected partner had genital HIV-1 levels below the limit of quantification. Plasma HIV-1 was detectable for all 11 persons with undetectable genital HIV-1 concentrations who transmitted HIV-1 to their partners. The reason for this could be that a single assessment of genital HIV-1 burden may miss intermittent shedding of genital virus (*16*, *25*).

In our study, as in multiple previous studies, plasma and genital HIV-1 concentrations were only modestly correlated (*1*). We found that genital HIV-1 concentrations remained independently associated with HIV-1 transmission risk after adjustment for plasma HIV-1 levels, as well as other clinical and behavioral factors. Genital HIV-1 levels display greater variability than do plasma HIV-1 levels (*13*); greater variability in the measurement of genital versus plasma HIV-1 would not alter the accuracy of our findings (that is, the point estimate of risk of HIV-1 transmission versus $\log_{10}$ genital HIV-1 levels) but would contribute to the precision of the estimate (that is, the width of the CIs). Recent work suggests that genital HIV-1 levels, like those in plasma, establish a relatively stable set point after acute infection (*18*). Thus, a single measurement, as done in this study, may provide a useful biomarker of HIV-1 infectiousness, particularly given the challenges of obtaining repeat genital HIV-1 measurements in large studies. HIV-1 replication may be different at genital mucosal sites compared to other sites that contribute virus to the blood, potentially because of genital tract infections or local immunological factors (*1*, *18*, *26*–*29*). Thus, genital HIV-1 levels, as potentially the most relevant and proximate marker of HIV-1 exposure for sexual HIV-1 transmission, may predict HIV-1 risk as well as or better than plasma HIV-1 concentrations alone. We found that only genital HIV-1 levels in men were statistically related to HIV-1 transmission risk in a model that included both genital and plasma HIV-1 RNA concentrations, whereas for women both blood plasma and genital HIV-1 RNA were independently predictive. These findings could reflect the biology of menses and the contribution of blood HIV-1 to the female genital tract, which is not a consideration for men. More limited statistical power for our analysis of male-to-female HIV-1 transmission (given a smaller number of HIV-1–infected men compared to women in our study population) may also explain these findings. Future studies of genital HIV-1 should explore characteristics of those variants that are transmitted, including genetic sequence differences, viral fitness, and whether the source of transmitted virus is cell-free or cell-associated HIV-1 (*1*, *30*).

We found that acyclovir reduced genital HIV-1 levels by ~0.3 $\log_{10}$, a result that was statistically significant and similar to previous studies of HSV-2–suppressive therapy (*13*); in our trial, acyclovir reduced plasma HIV-1 levels by 0.25 $\log_{10}$ copies/ml but did not reduce HIV-1 transmission (*19*). We recently estimated that a nearly 0.75 $\log_{10}$ copies/ml reduction in plasma HIV-1 RNA would be necessary to decrease HIV-1 transmission by 50% (*24*). Thus, interventions that greatly reduce HIV-1 levels, like ART, are likely to have more substantial effects on HIV-1 transmission risk than interventions that reduce HIV-1 concentrations minimally.

We only collected one sample per study participant for genital HIV-1 quantification. Repeat measurements might have increased precision in our regression estimates because the variability in HIV-1 concentrations is greater in genital samples than in plasma samples (*2*). However, despite this potential for improvement in analytical precision, we still observed a strong relationship between genital HIV-1 levels and HIV-1 transmission risk. Previous studies of genital HIV-1 have collected a single or a small number of genital samples per individual to measure the effect on genital HIV-1 shedding of interventions aimed at decreasing HIV-1 infectiousness, including HSV-2–suppressive therapy (*9*, *11*), treatment of curable sexually transmitted infections (*7*, *8*, *22*), and initiation of ART (*15*). Our results confirm that a single measurement of genital HIV-1 quantity is a strong surrogate marker of HIV-1 transmission risk, and suggest that the potential impact of new interventions aimed at reducing HIV-1 transmission can be assessed through studies of genital HIV-1 RNA. With >2500 participants, this is the largest study of genital HIV-1 in African persons.

A limitation of this study is that some HIV-1 transmission events occurred before or several months after collection of the genital sample. However, the median time from acquiring the genital sample to HIV-1 seroconversion was less than 6 months, and for most HIV-1 transmission events, the genital sample was collected before or at the time of seroconversion. Sensitivity analyses assessing the timing of genital sample collection relative to HIV-1 transmission and the collection of a plasma sample for HIV-1 RNA quantification generated results similar to those from the analysis of all participants. Etiological screening for sexually transmitted infections was done at study enrollment and not when genital HIV-1 RNA samples were collected. Finally, HIV-1–infected partners were also HSV-2 seropositive. HSV-2 is common among persons with HIV-1 (seroprevalence, 50 to 90%) (*31*), and thus, this is unlikely to limit the generality of our findings.

Understanding the relationship between genital HIV-1 replication and the risk of HIV-1 transmission is central to describing the fundamental biological mechanisms underlying HIV-1 transmission. ART

and other potential new interventions such as HIV-1 vaccines that reduce systemic and genital HIV-1 replication, and interventions that reduce genital HIV-1 concentrations alone (such as treatment of genital tract infections and antiretroviral-based microbicides) should continue to be evaluated for their potential to reduce HIV-1 transmission. Genital sampling should be used to quantify the potential reduction in HIV-1 transmission risk of interventions that are directed at reducing the infectiousness of persons with HIV-1.

MATERIALS AND METHODS

Population and procedures

Between November 2004 and April 2007, heterosexual HIV-1 serodiscordant couples were enrolled from 14 sites in seven African countries (Botswana, Kenya, Rwanda, South Africa, Tanzania, Uganda, and Zambia) in a randomized, placebo-controlled, clinical trial of HSV-2 daily suppressive acyclovir therapy for prevention of HIV-1 transmission (clinicaltrials.gov number NCT00194519) (*32*). Follow-up was for up to 24 months per couple; some couples were followed for less than 24 months because of scheduled study closure. All study follow-up was completed by October 2008. HSV-2–suppressive therapy, provided to the HIV-1–infected members of the couples, failed to reduce HIV-1 transmission, despite an average 0.25 $\log_{10}$ copies/ml reduction in plasma HIV-1 levels (*19*).

Couples were eligible if both members were ≥18 years of age and if they reported three or more episodes of vaginal intercourse during the 3 months before enrollment. HIV-1–infected partners were HIV-1 and HSV-2 seropositive, were not using ART, and had a $CD4^+$ T cell count of ≥250 cells/mm^3 and no history of AIDS-defining conditions.

HIV-1–infected partners were seen monthly. At the time the study was conducted, national guidelines generally recommended ART initiation at $CD4^+$ T cell counts of less than 200 to 250 cells/mm^3 or in persons with clinical AIDS. HIV-1–infected persons who met national guidelines for initiation of ART during follow-up, as a result of $CD4^+$ T cell decline or change in clinical status, were referred to local HIV-1 care clinics to start ART. HIV-1–infected men provided one semen sample for HIV-1 RNA quantification at any visit ≥3 months after enrollment. HIV-1–infected women underwent a speculum pelvic examination at a visit 6 months after enrollment, during which an endocervical Dacron swab for HIV-1 RNA quantification was obtained; swabs were not collected at a defined time in the menstrual cycle, although women usually deferred sampling when they were menstruating. HIV-1–uninfected partners were seen quarterly for risk assessment and tests for HIV-1 antibodies.

All participants received pre- and posttest HIV-1 counseling, risk reduction counseling (individually and as a couple), free condoms, and treatment of sexually transmitted infections according to World Health Organization (WHO) guidelines. The study protocol was approved by the University of Washington Human Subjects Review Committee and ethical review committees at each collaborating organization. All participants provided written informed consent.

Laboratory analyses

HIV-1 serological testing was by dual rapid HIV-1 antibody tests, with positive results confirmed by HIV-1 enzyme immunoassay (EIA) and Western blot. For HIV-1 seroconverters, HIV-1 transmission endpoints were classified as either "genetically linked" within the partnership or "unable to be linked" (that is, likely acquired outside of the study partnership), based on sequencing of HIV-1 C2-V3-C3 regions of *env* and p17/p24 regions of *gag* amplified from plasma from the seroconverting partner and the HIV-1–infected partner with whom they enrolled in the study. Phylogenetic analysis and posterior probability of linkage using pairwise nucleotide distances between sequences were performed as previously detailed (*19*).

HSV-2 serostatus was determined by HSV Western blot (*33*). At study conclusion, batched testing of enrollment samples included nucleic acid amplification for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* of endocervical swab or urine samples (Gen-Probe) (*32*).

$CD4^+$ T cell quantification was performed for HIV-1–infected participants every 6 months with flow cytometry. HIV-1 RNA was quantified from plasma at baseline; at months 3, 6, and 12; and at study exit. HIV-1 RNA was quantified from seminal plasma and endocervical swabs with the COBAS AmpliPrep/COBAS TaqMan real-time HIV-1 RNA assay version 1.0 (Roche Diagnostics) (*19*). The assay was validated for seminal plasma and fluid eluted from endocervical swabs with HIV-1–spiked Virology Quality Assurance Program standards and published specimen-processing procedures (*11*, *13*). Endocervical swabs were eluted in 1000 μl of GUSCN lysis buffer, eluted for 15 min, vortexed briefly, and microfuged for 5 s at 14,000*g* to pellet debris before removal of fluid for testing. A final dilution step with 10× phosphate-buffered saline (PBS) was used to achieve sufficient volume for the COBAS AP/TM assay, with a lower limit of quantification of 240 copies (per milliliter for blood plasma and seminal plasma and per swab for endocervical samples). Validation of the COBAS assay against an independently validated quantitative HIV-1 real-time polymerase chain reaction (PCR) assay showed assay precision of <0.24 $\log_{10}$ copies/ml, which was not significantly different between the two assay platforms (*11*, *13*). Assay inhibitors were removed by the COBAS AmpliPrep procedure.

Data analysis

Plasma and genital HIV-1 RNA concentrations were $\log_{10}$-transformed to approximate normality. Samples below the limit of quantification were assigned values at half that limit. Genital HIV-1 RNA concentrations and male-to-female and female-to-male transmission were analyzed separately.

Couples in which the HIV-1–infected participant contributed a genital sample for HIV-1 RNA quantification were included in this analysis (*19*). The primary outcome measure was detection of HIV-1 seroconversion, and we restricted analyses to genetically linked HIV-1 transmissions. Participants with genetically unlinked HIV-1 transmissions contributed follow-up time until HIV-1 seroconversion; data from subsequent visits were censored. Couples in which the HIV-1–infected partners initiated ART were censored at ART initiation, and genital samples collected subsequently were not analyzed (n = 43). Twenty couples were excluded because semen samples had insufficient volume for testing.

We used Cox proportional hazards analysis to assess the relationship between genital HIV-1 concentrations and risk of genetically linked HIV-1 seroconversion among initially HIV-1 seronegative partners. Acyclovir did not reduce the risk of HIV-1 transmission, but significantly reduced plasma HIV-1 RNA levels by an average of 0.25 $\log_{10}$ copies/ml (*19*); thus, analyses were stratified by randomization

arm. To account for potential unmeasured differences across the 14 sites, we also stratified analyses by study site. Analyses were adjusted for plasma HIV-1 RNA levels as a time-dependent variable and for other potential correlates of HIV-1 transmission, including unprotected sex, sexually transmitted infections, HSV-2 serostatus of the HIV-1–uninfected partner, $CD4^+$ T cell count of the HIV-1–infected partner, circumcision status of the male partner, and age. Given that genital samples were collected only once during the study, genital HIV-1 RNA quantity was analyzed as a time-independent variable, with follow-up time beginning at enrollment; sensitivity analyses were performed to assess the effect of timing of genital sample collection. χ^2 and Mann-Whitney *U* tests were used to compare categorical and continuous characteristics, respectively. Spearman's correlation coefficient and linear regression were used to assess the relationship between genital and plasma HIV-1 concentrations. Data were analyzed with SAS version 9.20.

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