MAJOR ARTICLE

Determinants of Per-Coital-Act HIV-1 Infectivity Among African HIV-1–Serodiscordant Couples

James P. Hughes,[1,5] Jared M. Baeten,[2,3,9] Jairam R. Lingappa,[2,3,4] Amalia S. Magaret,[4,10] Anna Wald,[2,9] Guy de Bruyn,[6] James Kiarie,[7] Mubiana Inambao,[8] William Kilembe,[8] Carey Farquhar,[2,9] Connie Celum,[2,3,9] and the Partners in Prevention HSV/HIV Transmission Study Team

[1]Department of Biostatistics, [2]Department of Medicine, [3]Department of Global Health, and [4]Department of Pediatrics, University of Washington, Seattle; [5]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington; [6]Perinatal HIV Research Unit, University of Witwatersrand, Johannesburg, Republic of South Africa; [7]Department of Obstetrics and Gynecology, University of Nairobi and Kenyatta National Hospital, Kenya; [8]Rwanda-Zambia HIV Research Group (RZHRG), Ndola, Zambia; [9]Department of Epidemiology and [10]Department of Laboratory Medicine, University of Washington, Seattle

(See the editorial commentary by Gray and Wawer on pages 351–2.)

Background. Knowledge of factors that affect per-act infectivity of human immunodeficiency virus type 1 (HIV-1) is important for designing HIV-1 prevention interventions and for the mathematical modeling of the spread of HIV-1.

Methods. We analyzed data from a prospective study of African HIV-1–serodiscordant couples. We assessed transmissions for linkage within the study partnership, based on HIV-1 sequencing. The primary exposure measure was the HIV-1–seropositive partners' reports of number of sex acts and condom use with their study partner.

Results. Of 3297 couples experiencing 86 linked HIV-1 transmissions, the unadjusted per-act risks of unprotected male-to-female (MTF) and female-to-male (FTM) transmission were 0.0019 (95% confidence interval [CI], .0010–.0037) and 0.0010 (95% CI, .00060–.0017), respectively. After adjusting for plasma HIV-1 RNA of the HIV-1–infected partner and herpes simplex virus type 2 serostatus and age of the HIV-1–uninfected partner, we calculated the relative risk (RR) for MTF versus FTM transmission to be 1.03 ($P = .93$). Each $\log_{10}$ increase in plasma HIV-1 RNA increased the per-act risk of transmission by 2.9-fold (95% CI, 2.2–3.8). Self-reported condom use reduced the per-act risk by 78% (RR = 0.22 [95% CI, .11–.42]).

Conclusions. Modifiable risk factors for HIV-1 transmission were plasma HIV-1 RNA level and condom use, and, in HIV-1–uninfected partners, herpes simplex virus 2 infection, genital ulcers, *Trichomonas vaginalis*, vaginitis or cervicitis, and male circumcision.

Human immunodeficiency virus type 1 (HIV-1) infectivity is defined as the probability of transmission per coital act with an infected partner. Knowledge of HIV-1 infectivity and factors that affect it are important for patient counseling, design of prevention interventions, and the mathematical modeling of the spread of disease. Infectivity can be estimated from prospective HIV-1 serodiscordant partner studies, although such studies are technically challenging to conduct [1]. In particular, obtaining accurate counts of sex acts during the interval between HIV-1 tests is difficult, especially if the interval is long. In addition, failure to identify and eliminate transmissions from outside the study partnership may lead to inflated estimates of infectivity and misclassification of risk factors. Measuring cofactors of transmission risk, particularly those that change over time (eg, sexual behavior or plasma HIV-1 RNA concentration), is also difficult, and assessing the effect of multiple cofactors on transmission requires large sample sizes.

A recent meta-analysis [2] reviewed 26 studies that estimated HIV-1 infectivity. The pooled estimates of male-to-female (MTF) and female-to-male (FTM) transmission in low-income settings were 0.0030 and 0.0038 per act, respectively. However, in 14 studies that followed

Received 5 April 2011; accepted 9 August 2011; electronically published 12 January 2012.

Presented in part: 18th Conference on Retrovirology and Opportunistic Infections, Boston, 27 February–2 March 2011. Paper 135.

Correspondence, James P. Hughes, PhD, Department of Biostatistics, University of Washington 357232, Seattle, WA 98195-7232 (jphughes@u.washington.edu).

The Journal of Infectious Diseases 2012;205:358–65

DOI: 10.1093/infdis/jir747

serodiscordant couples, the median number of couples was only 73, and only 4 of these studies [3–6] included >200 couples. The ability of most studies to examine cofactors of transmission was, therefore, limited. Last, only a few studies [4, 6, 7] have evaluated the role of plasma HIV-1 RNA on infectivity, and only 2 studies [4, 6] have used molecular confirmation to determine linkage of transmissions [8].

In this study, we analyze data from a longitudinal cohort of >3400 African HIV-1–serodiscordant heterosexual couples. We estimate the infectivity of HIV-1 and evaluate factors that affect infectivity. Importantly, and unique to this study, these data include repeated measurements of plasma HIV-1 RNA in the HIV-1–infected partner and molecular confirmation to determine linkage of all transmissions.

METHODS

We used data from the Partners in Prevention Herpes Simplex Virus (HSV)/Human Immunodeficiency Virus (HIV) Transmission Study, a randomized clinical trial of HSV-2 suppressive therapy for prevention of HIV-1 transmission (ClinicalTrials.gov NCT00194519). Stable, HIV-1–serodiscordant couples in which the HIV-positive partner was also infected with HSV-2 were enrolled at 14 sites in eastern and southern Africa and followed for ≤24 months. The primary objective of the trial was to evaluate the efficacy of daily acyclovir HSV-2–suppressive therapy for preventing HIV-1 transmission. No significant difference in transmission risk was seen between the intervention and control groups. The design, methods, and primary outcomes have been described previously [9, 10].

Among the 3408 enrolled couples, confirmatory testing at the end of the trial found that 27 partners initially categorized as "HIV-1 infected" did not meet the HIV-1 (n = 3) or HSV-2 (n = 24) serologic eligibility criteria. An additional 24 couples were excluded from this analysis after the HIV-1–uninfected partner was determined to have been HIV-1 positive at enrollment by retrospective polymerase chain reaction (PCR) testing. Finally, 60 couples provided no postenrollment HIV-1 test data. Thus, 3297 couples were included in this analysis.

At enrollment, demographic data were collected on each partner, participants were tested for sexually transmitted infections (STIs), men were examined to determine circumcision status, and the HIV-1–uninfected partner was tested to determine HSV-2 serostatus. At quarterly visits, each participant underwent a genital examination and the uninfected partner was tested for HIV-1 seroconversion. Participants received comprehensive HIV-1 prevention measures, including HIV-1 risk-reduction counseling (both individually and as a couple), quarterly syndromic STI treatment, and free condoms.

Plasma HIV-1 RNA in the infected partner was measured at enrollment, 3, 6, and 12 months, and study exit (typically, 24 months). We used the most recent viral load prior to or concurrent with each HIV-1 test as a time-varying covariate in our analyses, except that prior viral loads were not carried forward if antiretroviral therapy was started.

Information on the number of sex acts during follow-up comes from 2 sources: HIV-1–infected partners were interviewed monthly, and they provided information on the numbers of acts with the study partner, with and without a condom, since their last visit (by protocol, 1 month prior). The total reported number of sex acts with the study partner between HIV-1 tests (by protocol, 3 months)—our primary exposure measure—was obtained by summing data over all the monthly visits of the HIV-1–infected partner in the testing interval. Because the 2 partners' visits did not always coincide exactly, the reported number of acts in the testing interval was scaled by a factor equal to the length of the testing interval divided by the number of days covered by the HIV-1–infected partner's reports. For example, if the interval between HIV-1 tests was 90 days, but the HIV-1–infected partner's behavioral data covered only 60 days in that interval, then the reported number of acts was increased by a factor of 90 divided by 60. A secondary source of information was provided by the HIV-1–uninfected partners who were asked about their number of acts (with study partner, with or without condom) over the month preceding each HIV-1 test. These data were not used directly in the analyses but were used to help evaluate the impact of mismeasurement of the number of acts (see Appendix; supplementary data). Because HIV-1 serostatus was not available from nonstudy partners, acts with these partners were not included in any analyses.

The protocol was approved by the University of Washington Human Subjects Review Committee and ethical review committees at local and collaborating organizations. Participants provided written informed consent.

Lab Methods

During follow-up, the HIV-1–uninfected partners were tested quarterly by HIV-1 rapid tests; positive results were confirmed by Western blotting. After the study was complete, preseroconversion plasma samples were tested for HIV-1 RNA by PCR to more precisely determine the timing of infection; the time of HIV-1 infection was defined as the earlier of positive serology or PCR. Plasma HIV-1 RNA was quantified using the COBAS TaqMan real-time HIV-1 RNA assay, version 1.0 (Roche Diagnostics).

Each confirmed HIV-1 transmission was classified as "linked" (transmission between study partners) or "unlinked" (HIV-1 acquisition from a nonstudy partner) on the basis of sequencing of plasma samples from the source and infected partner for the C2-V3-C3 regions of *env* and the p17/p24 regions of *gag*, phylogenetic analysis, and posterior probability of linkage using pairwise nucleotide distances between sequences [8]. Only linked transmissions are included in this analysis; couples with

unlinked transmissions were censored from the analysis at the time of HIV-1 infection.

Serologic testing for HSV-2 and nucleic acid amplification testing for STIs (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*) were done at study enrollment, as previously described [11]. Syphilis serology was performed at enrollment using rapid plasma reagin with confirmation by the microhemagglutination test for *Treponema pallidum* at sites with that capacity. During follow-up, genital ulcers and other STI symptoms (eg, urethritis or vaginal discharge) were evaluated by history in the prior 30 days and on quarterly exams.

Statistical Methods

Jewell and Shiboski [12] relate per-act infectivity (λ), number of acts over the interval between HIV-1 tests (n), and covariates (X) to the probability of HIV-1 transmission from the infected partner to the uninfected partner, $p(X;n)$, using the model:

$$p(X;n) = 1-(1-\lambda)^{ne^{X\beta}} \quad (1)$$

For small values of λ, β may be interpreted as the log relative risk of infectivity for each unit change in X. We modified model (1) to

$$p(X;n) = 1-(1-\lambda_0)^{n_0 e^{X\beta}}(1-\lambda_1)^{n_1 e^{X\beta}} \quad (2)$$

where n_0 acts are unprotected and n_1 acts are protected, λ_0 and λ_1 are the infectivities without and with a condom, respectively, and β represents the log relative risks. We used maximum likelihood methods (Stata procedure ml) to estimate the parameters of model (2), test hypotheses, and form confidence intervals.

For comparability to previous studies, we fit a model that included only gender and condom use (yes/no, for each act) to estimate the per-act risk of unprotected MTF and FTM transmission. To assess the impact of other covariates on infectivity, we first fit a base model that included plasma HIV-1 RNA concentration and condom use. Randomization group, demographic factors (gender, age, partnership duration at enrollment), time on study, STIs (by lab test at baseline and syndromic diagnoses over follow-up), HSV-2 in the HIV-1–uninfected partner, and circumcision status of the male partner were added to the base model individually, with separate terms for the HIV-1–infected and the HIV-1–uninfected partner, where appropriate. We included in a multivariate model all covariates that were significant at $P < .1$ when added to the base model, and we used backward elimination (eliminating terms that were not significant at the $P = .05$ level) to develop a final model. All reported P values are 2-sided. Analyses were performed using Stata 11 software [13].

We investigated heterogeneity in λ (beyond that which can be explained by the covariates X) by including a random intercept in the linear predictor ($X\beta$) in model (1) [12].

RESULTS

At enrollment, HIV-1–infected partners were primarily female (67%) and their median age was 32 years (interquartile range [IQR], 26–38 years); 34% of HIV-1–infected males were circumcised (Table 1). The median plasma HIV-1 RNA concentration at enrollment was 3.91 $\log_{10}$ copies/mL (IQR, 3.16–4.53 $\log_{10}$ copies/mL). HIV-1–uninfected partners were slightly older (median, 33 [IQR, 28–40]), 68% were HSV-2 seropositive, and 55% of men were circumcised. HIV-1–infected partners had genital herpes recurrences (genital ulcer disease [GUD]) on exam or self-reported symptoms in the prior interval) at 9.2% of quarterly follow-up visits and HIV-1–uninfected partners had GUD on exam or by self-report at 5.2% of quarterly follow-up visits.

The median number of unprotected and protected acts over the preceding 30 days, as reported by the HIV-1–infected partners at enrollment, was 0 (IQR, 0–1) and 3 (IQR, 1–5), respectively. Over follow-up, the median rate of unprotected and protected acts per 30 days, as reported by the HIV-1–infected partners, was 0 (IQR, 0–0) and 3.3 (IQR, 1.8–5.9), respectively, and 93% of sex acts were reported as protected. The median total number of sex acts per 30 days declined steadily from 4.0 at enrollment to 2.5 by month 24.

Overall, 86 linked transmission events were observed during follow-up. Table 2 shows the relationship between total number of reported acts within a testing interval and the HIV-1 test result at the end of that interval. There were 3 transmissions (3.5%) in which the HIV-1–infected partner reported 0 acts in the interval immediately prior to a linked infection (although in 1 of these cases, the report only covered a portion of the interval). In none of these cases did the HIV-1–uninfected partner report sex acts with anyone other than their study partner. These 3 transmissions cannot be included in estimates of infectivity as they lead to an infinite likelihood in the statistical analysis.

In a model that included only condom use and gender, the estimated risks of unprotected MTF and FTM transmission were 0.0019 (95% CI, .0010–.0037) and 0.0010 (95% CI, .00060–.0017), respectively (relative risk [RR] = 1.95; $P = .003$). However, after adjustment for plasma HIV-1 RNA and HSV-2 status and age of the uninfected partner (all of which differed significantly depending on the gender of the HIV-1–infected partner), the RR for MTF transmission was attenuated to 1.03 ($P = .93$), suggesting that the higher risk of MTF transmission was largely due to higher viral loads in men (over follow-up, mean viral load measurement in men = 4.1 $\log_{10}$ copies/mL; in women = 3.8 $\log_{10}$ copies/mL) and other sources of confounding.

Log_{10} plasma HIV-1 RNA was entered linearly into model (2). A more complex functional form using cubic splines did not significantly improve the fit ($P = .2$, comparing the linear model to the spline model). Figure 1 shows the relationship between infectivity and $\log_{10}$ plasma HIV-1 RNA in a model that includes

Table 1. Characteristics of the 3297 Couples

	HIV-1–Infected Partner	
	Male	Female
Baseline	n = 1074	n = 2223
Age, y	37 (32–45)	30 (25–34)
Plasma HIV-1 RNA, log_{10} copies/mL	4.2 (3.4–4.8)	3.8 (3.0–4.4)
CD4 count, cells/mL		
250–349	315 (30%)	537 (24%)
350–499	377 (35%)	646 (29%)
>500	379 (35%)	646 (29%)
Circumcised	363 (66%)	…
Chlamydia trachomatis[a]	12 (1.1%)	53 (2.6%)
Neisseria gonorrhoeae[a]	8 (0.8%)	39 (1.9%)
Trichomonas vaginalis[a]	49 (4.6%)	353 (17%)
Syphilis seropositive[ab]	69 (6.4%)	161 (7.2%)
Quarterly follow-up	n = 6475 visits	n = 12,966 visits
Plasma HIV-1 RNA (log_{10} copies/mL)	4.3 (3.5–4.9)	3.9 (3.1–4.6)
Antiretroviral use	366 (6.0%)	543 (4.5%)
Genital ulcerative disease, on exam or by report	418 (6.9%)	774 (6.4%)
Urethitis[a]	37 (0.6%)	…
Vaginitis or cervicitis[a]	…	944 (8.9%)
Sex acts with reported condom use	96.6%	91.6%
	HIV-1–Uninfected Partner	
Baseline	n = 2223	n = 1074
Age, y	35 (29–42)	30 (25–37)
Partnership duration, y	5.0 (2.1–9.4)	6.7 (3.0–13.8)
Circumcised	1225 (55%)	…
HSV-2 seropositive[ab]	1318 (59%)	927 (86%)
C. trachomatis[ab]	63 (2.9%)	18 (1.9%)
N. gonorrhoeae[ab]	10 (0.5%)	10 (1.1%)
T. vaginalis[ab]	149 (6.8%)	115 (12%)
Syphilis seropositive[ab]	117 (5.3%)	44 (4.1%)
Quarterly follow-up	n = 12,966 visits	n = 6475 visits
GUD on exam or by report	501 (3.9%)	345 (5.3%)
Urethitis[a]	123 (1.0%)	…
Vaginitis or cervicitis[a]	…	366 (6.6%)
Visit at which HIV-1 transmission occurred, mo after enrollment		
3	9	12
6	6	10
9	5	9
12	5	7
15	6	4
18	5	3
21	1	1
24	2	1

Medians (interquartile ranges) are presented for quantitative measures; counts (%) are presented for categorical measures. Percents are computed as a fraction of nonmissing observations.

Abbreviations: GUD, genital ulcer disease; HIV-1, human immunodeficiency virus type 1; HSV-2, herpes simplex virus type 2; y, years.

[a] Results are counts (%) positive for each infection/condition.

[b] CT, GC and TV by NAAT, and HSV-2 by Focus EIA confirmed by WB, and syphilis by TPHA.

plasma HIV-1 RNA and reported condom use only. Each log_{10} increase in plasma HIV-1 RNA increases the per-act risk of transmission by a factor of 2.89 so that the estimated per act risk of transmission without a condom at 3, 4, 5, and 6 logs is 0.00028, 0.00082, 0.0024, and 0.0068, respectively.

Table 3 shows the RR, overall and by gender, for characteristics of the HIV-1–infected and HIV-1–uninfected partner in univariate analyses. In a multivariate model (Table 4), plasma HIV-1 RNA and condom use reported by the HIV-1–infected partner and age, HSV-2 serostatus, GUD by exam or self-report, *T. vaginalis* (at enrollment), cervicitis or vaginitis (during follow-up), and male circumcision status of the HIV-1–uninfected partner remained significant. Circumcision in male HIV-1–uninfected partners was associated with significantly lower infectivity (RR, 0.53 [95% CI, .29–.96]), and infectivity also declined as the age of the HIV-1–uninfected partner increased (RR, 0.82 per 5-year increase [95% CI, .71–.94]). We found similar results when the age of the HIV-1–infected partner was substituted for that of the uninfected partner in the model. Condom use reduced infectivity by 78% (RR, 0.22 [95% CI, .11–.42]). However, 56 linked transmissions occurred in intervals in which all acts were reported to be protected. The protective effects of reported condom use was similar regardless of whether the HIV-1–infected partner was male (RR, 0.14) or female (RR, 0.29) (P value for gender by condom interaction = 0.29). HSV-2 seropositivity (RR, 2.14; [95% CI, 1.18–3.88]), GUD by exam or self-report (RR, 2.65 [95% CI, 1.35–5.19]), *T. vaginalis* infection at enrollment (RR, 2.57 [95% CI, 1.42–4.65]) and a clinical diagnosis of vaginitis or cervicitis during follow-up (RR, 3.63 [95% CI, 1.47–8.92]) in the HIV-1–uninfected partner were independently associated with an elevated per-act risk of transmission. Characteristics of the HIV-1–infected partner (including recurrent genital herpes by exam or self-report, *T. vaginalis* positivity, antiretroviral agent use, and circumcision status), presence of other curable STI (*C. trachomatis, T. pallidum*) in either partner, urethritis in male partners, partnership duration, and time on study were not significant in the multivariate analysis ($P > .20$).

We also found evidence of additional unexplained heterogeneity in infectivity—the addition of a random effect for infectivity significantly improved the fit ($P = .005$; data not shown). This suggests that there are unmeasured viral, host, or behavioral factors that induce additional variation in infectivity among couples; inaccurate reporting of the number of acts and condom use may also contribute to unexplained heterogeneity.

DISCUSSION

In this prospective study of 3297 African HIV-1 discordant couples, we found unadjusted per-act risks of unprotected MTF and FTM transmission of 0.0019 and 0.001, respectively. However, after adjustment for plasma HIV-1 RNA levels and the

Table 2. Total Number of Acts (With and Without a Condom) and Transmissions, by Gender of the HIV-1–Infected Partner, Within Testing Intervals

	Female HIV-1–Infected Partner, Total No. of Acts						
	0	1–5	6–10	11–20	20–40	>40	…
Intervals with no transmission	1200	2445	3097	3283	1942	567	385
Intervals in which transmission occurred	1	8	14	11	11	1	1
Proportion of intervals resulting in transmission	0.0008	0.0033	0.0034	0.0033	0.0056	0.0018	0.0026
	Male HIV-1–Infected Partner, Total No. of Acts						
	0	1–5	6–10	11–20	20–40	> 40	…
Intervals with no transmission	438	1209	1560	1851	1012	210	156
Intervals in which transmission occurred	2	6	15	10	3	3	0
Proportion of intervals resulting in transmission	0.0045	0.0049	0.0095	0.0064	0.0030	0.0141	0.0

By protocol, intervals were 90 days.

Abbreviation: HIV-1, human immunodeficiency virus type 1.

HIV-1–uninfected partner's HSV-2 serostatus and age, the relative risk of MTF versus FTM transmission was almost 1.0, suggesting that much of the gender difference in infectivity could be explained by higher plasma HIV-1 RNA in HIV-1–infected men than women and other gender-related differences. Our unadjusted per-act risks are somewhat lower than the meta-analytic results of Boily et al for low-income settings [2], similar to those reported by Wawer et al [6] (when MTF and FTM transmissions are combined), and higher than the metaestimate for Africa reported by Powers et al [1]. Similarly to the approach of Wawer et al [6], we restricted our analysis to molecularly confirmed transmissions. Several gender-specific factors also significantly influenced the per-act risk of HIV-1 transmission, including circumcision status of the HIV-1–uninfected male partner (associated with reduced susceptibility), *T. vaginalis* infection in the HIV-1–uninfected female partner at enrollment (associated with increased susceptibility), and cervicitis or vaginitis in the female HIV-1–uninfected partner at the HIV-seroconversion visit (also associated with increased susceptibility). However, because symptoms of cervicitis or vaginitis and diagnosis of HIV-1 infection occurred in the same interval, the relative timing of cervicitis or vaginitis with HIV-1 seroconversion could not be ascertained. After adjustment for plasma HIV-1 RNA levels, antiretroviral agent use in the HIV-1–infected partner did not significantly predict transmission.

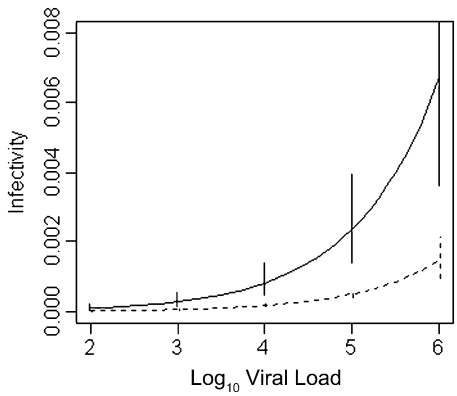

Figure 1. Per-act probability of transmission (infectivity) vs $\log_{10}$ plasma HIV-1 RNA (copies/mL) from a model that includes plasma human immunodeficiency virus type 1 RNA and condom use only. Solid line is without reported condom use and dashed line is with reported condom use. Vertical lines represent 95% confidence intervals.

This study is the first to estimate HIV-1 infectivity after adjusting for time-varying plasma HIV-1 RNA. Over a relatively broad range of plasma HIV-1 RNA levels (2–6 $\log_{10}$ copies/mL), we found that the relationship between log infectivity and $\log_{10}$ plasma HIV-1 RNA level was approximately linear and that each 10-fold increase in plasma HIV-1 RNA level was associated with a 2.9-fold increase in per-act transmission risk, underscoring the importance of a high plasma HIV-1 RNA level to HIV-1 transmission risk. We found no significant evidence of a "saturation effect" (a plasma HIV-1 RNA level above which infectivity does not increase), although only 1% of follow-up intervals and 3 transmissions occurred during periods with plasma HIV-1 RNA level of $\geq$6.0 $\log_{10}$ copies/mL.

We found that reported condom use decreased HIV-1 infectivity by 78%. This is consistent with a previous meta-analysis that reported an 80% protective effect of condoms [14]. Although the protective effect of condoms is expected, our results are unique because condom use was measured and analyzed on a per-act basis. In contrast to previous studies that reported low rates of condom use (eg, in Rakai [6]), <20% of couples reported occasional condom use and none reported consistent condom use), couples in this study reported that 93% of acts were protected and 100% condom use was reported in 82% of the intervals. Nonetheless, 56 transmissions occurred in intervals with 100% reported

Table 3. Relative Risks for Various Risk Factors in Univariate Analyses

	Gender of HIV-1–Infected Partner			
	Overall	Male	Female	*P* Value
Characteristics of HIV-1–infected partner				
Plasma HIV-1 RNA (per $\log_{10}$ copies/mL)	2.82 (2.16–3.69)	2.17 (1.46–3.27)	3.34 (2.32–4.82)	.11
Antiretroviral use	0.38 (0.05–2.74)	0[a] (…)	0.80 (0.11–5.78)	…
Reported condom use	0.24 (0.13–0.46)	0.13 (0.05–0.32)	0.26 (0.11–0.61)	.26
Age, per 5 y	0.88 (0.77–1.02)	0.72 (0.58–0.90)	0.89 (0.71–1.12)	.19
GUD by exam or self-report	0.51 (0.17–1.72)	0.84 (0.20–3.51)	0.32 (0.044–2.30)	.43
Circumcision (male)	…	0.55 (0.25–1.20)	…	…
Chlamydia trachomatis at enrollment	2.38 (0.75–7.56)	0[a] (…)	4.10 (1.27–13.3)	…
Treponema pallidum at enrollment	1.41 (0.65–3.07)	0[a] (…)	2.57 (1.15–5.75)	…
Trichomonas vaginalis at enrollment, female	…	…	1.10 (0.49–2.47)	…
Cervicitis or vaginitis during follow-up, female	…	…	1.81 (0.76–4.32)	…
Urethritis during follow-up, male	…	1.81 (0.76–4.32)	…	…
Characteristics of HIV-1–uninfected partner				
Partnership duration, per y	0.97 (0.94–1.01)	0.95 (0.90–1.00)	0.99 (0.93–1.04)	.33
Age, per 5 y	0.82 (0.71–0.94)	0.69 (0.54–0.88)	0.95 (0.80–1.12)	.04
HSV-2 seropositive at enrollment	2.05 (1.17–3.59)	0.98 (0.38–2.51)	2.38 (1.18–4.81)	.14
GUD by exam or self-report, during follow-up	2.88 (1.49–5.58)	2.04 (0.72–5.77)	3.60 (1.52–8.49)	.41
C. trachomatis at enrollment	1.67 (0.53–5.30)	1.54 (0.21–11.3)	1.79 (0.43–7.39)	.91
T. pallidum at enrollment	2.44 (1.22–4.88)	3.74 (1.46–9.60)	1.78 (0.64–4.97)	.30
T. vaginalis at enrollment, female	…	3.04 (1.35–6.83)	…	…
Cervicitis or vaginitis during follow-up, female	…	3.93 (1.60–9.60)	…	…
Urethritis during follow-up, male	…	…	3.24 (0.45–23.5)	…
Circumcision, male	…	…	0.53 (0.29–0.96)	…

Entries are relative risks (95% confidence intervals); the *P* value tests for a significant difference in relative risk between males and females.

Abbreviations: GUD, genital ulcer disease; HIV-1, human immunodeficiency virus type 1; HSV-2, herpes simplex virus type 2; y, years.

[a] Sparse data; relative risk is 0 but confidence interval cannot be computed.

condom use, suggesting that condom use was overreported and that the estimated effect of condom use may be attenuated compared with the true effect.

Previous studies have highlighted the increased risk of transmission associated with HSV-2 and GUD. In a meta-analysis, the presence or history of GUD in either partner was associated with

Table 4. Relative Risks in Per-Act Probability of HIV-1 Transmission

	Final Multivariate Model		
	RR	95% CI	*P* value
Characteristics of the HIV-1–infected partner[a]			
Plasma HIV-1 RNA during follow-up, per $\log_{10}$ copies/mL	2.89	2.19–3.82	<.001
Reported condom use during follow-up	0.22	.11–.42	<.001
Characteristics of the HIV-1–uninfected partner			
Age, per 5 y	0.82	.71–.94	.006
HSV-2 seropositive at enrollment	2.14	1.18–3.88	.012
GUD, by exam or self-report, during follow-up	2.65	1.35–5.19	.004
Trichomonas vaginalis at enrollment, female	2.57	1.42–4.65	.002
Cervicitis or vaginitis during follow-up, female	3.63	1.47–8.92	.005
Circumcision, male	0.53	.29–.96	.037

Abbreviations: CI, confidence interval; GUD, genital ulcer disease; HIV-1, human immunodeficiency virus type 1; HSV-2, herpes simplex virus type 2; RR, relative risk; y, years.

[a] Gender is included in the model to ensure interpretability of the sex-specific covariates.

a 5.3-fold increased risk of HIV-1 transmission [2]. We did not find an elevated risk of transmission associated with GUD by exam or self-report in the HIV-1/HSV-2 dually infected partner. However, we found a 2.14-fold increased risk of infection associated with HSV-2 positivity in the HIV-1–uninfected partner, which is similar to the 2- to 3-fold increased risk of HIV-1 acquisition associated with prevalent HSV-2 infection from meta-analyses [15, 16]. Also, similar to Powers et al [1], we found an independent 2.65-fold increased risk of infection associated with GUD by self-report or exam in HIV-1–uninfected partners.

The strengths of this study include the large number of couples, molecular confirmation of HIV-1 transmission between the study partners, repeated plasma HIV-1 RNA measurements over follow-up, and relatively short intervals of 3 months between HIV-1 tests. The study also had limitations. Because only couples that had not previously transmitted HIV-1 were enrolled in the study, if transmission risk varies significantly between couples, the highest-risk individuals would be expected to transmit early and never enter the cohort; such a "survivorship bias" could lead to an underestimate of infectivity. Supporting this possibility is the significant decline in infectivity with age and the evidence of additional unexplained heterogeneity in infectivity seen in the analysis of model fit. Nonetheless, no significant decline in infectivity was observed over follow-up in these data (after adjustment for covariates), and partnership duration was not a significant predictor of infectivity in our multivariate model. Enrolling stable discordant couples also meant that the likelihood of enrolling acute or recently infected partners was low, limiting our ability to assess the impact of HIV-1 stage on infectivity.

An important limitation of any infectivity study is the difficulty in measuring the number of sex acts in the interval between HIV-1 tests. Imprecision in act counts may arise from a number of sources. A 2- to 3-week lag between HIV-1 infection and detection may lead to imprecision in act counts over the relevant risk period. Also, we analyzed the number of acts reported by the HIV-1–infected partner, whose visit intervals did not always correspond precisely to the intervals between the HIV-1 tests of the HIV-1–uninfected partner. Finally, the observation that HIV transmissions occurred during intervals when the HIV-1–infected partner reported only protected sex indicates that condom use was overreported. Nonetheless, a simulation study (see Appendix; supplementary data) suggests that unbiased (with respect to condom use) misreporting of acts does not lead to significant bias in estimates of infectivity or the RR of other covariates and overreporting of condom use and underreporting of nonuse does bias estimates of infectivity (ie, the RR of condom use would be attenuated toward 1.0) but would have little effect on the RR of other covariates.

In summary, observed differences in MTF and FTM infectivity appear to be largely driven by measurable differences in plasma viral load of the HIV-1–infected partner, condom use, and HSV-2 status and age of the HIV-1–uninfected partner. Notably, after adjustment for plasma HIV-1 RNA, HSV-2 status, age, and condom use, we found no significant difference between MTF and FTM infectivity. This strong dependence of HIV-1 infectivity on cofactors suggests that the relationship of (unadjusted) MTF and FTM infectivity may vary substantially across different settings with different distributions of these key cofactors. HIV-1 infectivity increased log-linearly with $\log_{10}$ plasma HIV-1 RNA across the range of plasma HIV-1 RNA levels in this cohort (2–6 $\log_{10}$). Our results underscore the importance of >antiretroviral therapy [17, 18], and, possibly, treatment of coinfections [19, 20], to reduce plasma HIV-1 viral load in HIV-1–infected partners, and condom promotion, male circumcision, and treatment of symptomatic STIs for HIV-1–uninfected partners as potential interventions to reduce HIV-1 transmission.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We gratefully acknowledge the invaluable contributions of the HIV-1–serodiscordant couples that participated in this study. We thank the teams at the study sites and at the University of Washington for work on data and sample collection and management. We acknowledge Dr Renee Ridzon from the Bill & Melinda Gates Foundation for study oversight.

Financial support. This work was supported by funding from the Bill & Melinda Gates Foundation (26469) and the National Institutes of Health (AI029168 and AI083034).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Partners in Prevention HSV/HIV Transmission Study Team. University of Washington Coordinating Center and Central Laboratories, Seattle, WA: Connie Celum (principal investigator), Anna Wald (protocol cochair), Jairam R. Lingappa (medical director), Jared M. Baeten, Mary Campbell, Lawrence Corey, Robert W. Coombs, James P. Hughes, Amalia Magaret, M. Juliana McElrath, Rhoda Morrow, James I. Mullins.

Study sites and site principal investigators. Cape Town, South Africa (University of Cape Town): David Coetzee; Eldoret, Kenya (Moi University, Indiana University): Kenneth Fife, Edwin Were; Gaborone, Botswana (Botswana Harvard Partnership): Max Essex, Joseph Makhema; Kampala, Uganda (Infectious Disease Institute, Makerere University): Elly Katabira, Allan Ronald; Kigali, Rwanda (Rwanda Zambia HIV Research Group, and Emory University): Susan Allen, Kayitesi Kayitenkore, Etienne Karita; Kisumu, Kenya (Kenya Medical Research Institute, University of California, San Francisco): Elizabeth Bukusi, Craig Cohen; Kitwe, Zambia (Rwanda Zambia HIV Research Group, and Emory University): Susan Allen, William Kanweka; Lusaka, Zambia (Rwanda Zambia HIV Research Group, and Emory University): Susan Allen, Bellington Vwalika; Moshi, Tanzania (Kilimanjaro Christian Medical College, Harvard University): Saidi Kapiga, Rachel Manongi; Nairobi, Kenya (University of Nairobi, University of Washington): Carey Farquhar, Grace John-Stewart, James Kiarie; Ndola, Zambia (Rwanda Zambia HIV Research Group, and Emory University): Susan Allen, Mubiana Inambao; Orange Farm, South Africa

(Reproductive Health Research Unit, University of the Witwatersrand): Sinead Delany-Moretlwe, Helen Rees; Soweto, South Africa (Perinatal HIV Research Unit, University of the Witwatersrand): Guy de Bruyn, Glenda Gray, James McIntyre; Thika, Kenya (University of Nairobi, University of Washington): Nelly Rwamba Mugo.

Data management was provided by DF/Net Research (Seattle, WA), and site laboratory oversight was provided by Contract Lab Services (University of the Witwatersrand, Johannesburg, South Africa).

References

1. Powers KA, Poole C, Pettifor AE, Cohen MS. Rethinking the heterosexual infectivity of HIV-1: a systematic review and meta-analysis. Lancet Infect Dis **2008**; 8:553–63.
2. Boily MC, Baggaley RF, Wang L, et al. Heterosexual risk of HIV-1 infection per sexual act: systematic review and meta-analysis of observational studies. Lancet Infect Dis **2009**; 9:118–29.
3. Downs AM, De Vincenzi I. Probability of heterosexual transmission of HIV: relationship to the number of unprotected sexual contacts. European Study Group in Heterosexual Transmission of HIV. J Acquir Immune Defic Syndr Hum Retrovirol **1996**; 11:388–95.
4. Fideli US, Allen SA, Musonda R, et al. Virologic and immunologic determinants of heterosexual transmission of human immunodeficiency virus type 1 in Africa. AIDS Res Hum Retroviruses **2001**; 17:901–10.
5. Saracco A, Veglia F, Lazzarin A. Risk of HIV-1 transmission in heterosexual stable and random couples. The Italian Partner Study. J Biol Regul Homeost Agents **1997**; 11:3–6.
6. Wawer MJ, Gray RH, Sewankambo NK, et al. Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. J Infect Dis **2005**; 191:1403–9.
7. Gray RH, Wawer MJ, Brookmeyer R, et al. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. Lancet **2001**; 357:1149–53.
8. Campbell MS, Mullins JI, Hughes JP, et al. Partners in Prevention HSV/HIV Study Team. Viral linkage in HIV-1 seroconverters and their partners in an HIV-1 prevention clinical trial. PLoS One **2011**; 6:e16986.
9. Celum C, Wald A, Lingappa JR, et al. Partners in Prevention HSV/HIV Study Team. Acyclovir and transmission of HIV-1 from persons infected with HIV-1 and HSV-2. N Engl J Med **2010**; 362:427–39.
10. Lingappa JR, Baeten JM, Wald A, et al. Partners in Prevention HSV/HIV Study Team. Daily acyclovir for HIV-1 disease progression in people dually infected with HIV-1 and herpes simplex virus type 2: a randomised placebo-controlled trial. Lancet **2010**; 375:824–33.
11. Lingappa JR, Kahle E, Mugo N, et al. Partners HSV-2/HIV-1 Transmission Study Team. Characteristics of HIV-1 discordant couples enrolled in a trial of HSV-2 suppression to reduce HIV-1 transmission: the Partners Study. PLoS One **2009**; 4:e5272.
12. Jewell NP, Shiboski SC. Statistical analysis of HIV infectivity based on partner studies. Biometrics **1990**; 46:1133–50.
13. StataCorp. Stata: release 11. Statistical software. College Station, TX: StatCorp LP, **2009.**
14. Weller S, Davis K. Condom effectiveness in reducing heterosexual HIV transmission. Cochrane Database Syst Rev **2002**; 1. CD003255.
15. Wald A, Link K. Risk of human immunodeficiency virus infection in herpes simplex virus type 2–seropositive persons: a meta-analysis. J Infect Dis **2002**; 185:45–52.
16. Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. AIDS **2006**; 20:73–83.
17. Donnell D, Baeten JM, Kiarie J, et al. Partners in Prevention HSV/HIV Transmission Study Team. Heterosexual HIV-1 transmission after initiation of antiretroviral therapy: a prospective cohort analysis. Lancet **2010**; 375:2092–8.
18. Cohen MS, Chen YQ, McCauley M, et al. HPTN 052 Study Team. Prevention of HIV-1 infection with early antiretroviral therapy. New Engl J Med **2011**; 365:493–505.
19. Barnabas RV, Webb EL, Weiss HA, Wasserheit JN. The role of coinfections in HIV epidemic trajectory and positive prevention: a systematic review and meta-analysis. AIDS **2011**; 25:1559–73.
20. Modjarrad K, Vermund SH. An addition to the effect of treating coinfections on HIV-1 viral load. Lancet Infect Dis **2011**; 11:81.