Infectious Diseases

Risk of heterosexual HIV transmission attributable to sexually transmitted infections and non-specific genital inflammation in Zambian discordant couples, 1994–2012

Kristin M Wall,[1,2]* William Kilembe,[1] Bellington Vwalika,[1,3] Lisa B Haddad,[1,4] Eric Hunter,[1] Shabir Lakhi,[1,3] Roy Chavuma,[1,3] Naw Htee Khu,[1] Ilene Brill,[1,5] Cheswa Vwalika,[1] Lawrence Mwananyanda,[1] Elwyn Chomba,[1,6] Joseph Mulenga,[1] Amanda Tichacek[1] and Susan Allen[1]

[1]Department of Pathology & Laboratory Medicine, School of Medicine, Emory University, Atlanta, GA, USA, [2]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA, [3]Departments of Gynecology and Obstetrics (B.V.), Internal Medicine (S.L.) and Surgery (R.C.), School of Medicine, University of Zambia, Lusaka, Zambia, [4]Department of Gynecology and Obstetrics, Emory University, School of Medicine, Atlanta, GA, USA, [5]Department of Epidemiology, Ryals School of Public Health, University of Alabama at Birmingham, Birmingham, AL, USA and [6]Ministry of Community Development, Mother and Child Health, Lusaka, Zambia

*Corresponding author. 1518 Clifton Road NE, Atlanta, GA 30322, USA. E-mail: kmwall@emory.edu

Abstract

Background: Studies have demonstrated the role of ulcerative and non-ulcerative sexually transmitted infections (STI) in HIV transmission/acquisition risk; less is understood about the role of non-specific inflammatory genital abnormalities.

Methods: HIV-discordant heterosexual Zambian couples were enrolled into longitudinal follow-up (1994–2012). Multivariable models estimated the effect of genital ulcers and inflammation in both partners on time-to-HIV transmission within the couple. Population-attributable fractions (PAFs) were calculated.

Results: A total of 207 linked infections in women occurred over 2756 couple-years (7.5/100 CY) and 171 in men over 3216 CY (5.3/100 CY). Incident HIV among women was associated with a woman's non-STI genital inflammation (adjusted hazard ratio (aHR) = 1.55; PAF = 8%), bilateral inguinal adenopathy (BIA; aHR = 2.33; PAF = 8%), genital ulceration (aHR = 2.08; PAF = 7%) and the man's STI genital inflammation (aHR = 3.33; PAF = 5%), BIA (aHR = 3.35; PAF = 33%) and genital ulceration (aHR = 1.49; PAF = 9%). Infection among men was associated with a man's BIA (aHR = 4.11; PAF = 22%) and genital ulceration (aHR = 3.44; PAF = 15%) as well as with the woman's non-STI genital inflammation (aHR = 1.92; PAF = 13%) and BIA (aHR = 2.76; PAF = 14%). In HIV-M+F- couples, the

man being uncircumcised. with foreskin smegma. was associated with the woman's seroconversion (aHR = 3.16) relative to being circumcised. In F+M- couples, uncircumcised men with BIA had an increased hazard of seroconversion (aHR = 13.03 with smegma and 4.95 without) relative to being circumcised. Self-reporting of symptoms was low for ulcerative and non-ulcerative STIs.

Conclusions: Our findings confirm the role of STIs and highlight the contribution of non-specific genital inflammation to both male-to-female and female-to-male HIV transmission/acquisition risk. Studies are needed to characterize pathogenesis of non-specific inflammation including inguinal adenopathy. A better understanding of genital practices could inform interventions.

Key words: Couples' voluntary HIV counselling and testing, discordant couples, HIV risk, genital ulceration and inflammation, longitudinal cohort, Zambia

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Key Messages

- Observational research implicates several sexually transmitted infections (STIs) (including *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum* and herpes simplex virus 2), primarily as co-factors in HIV acquisition in high-risk women.
- Studies considering the risk conferred by more common genital inflammatory conditions not due to STI are lacking.
- Our findings confirm the contribution of STI and highlight the independent contribution of non-STI inflammatory processes to risk of HIV transmission and acquisition in both men and women.
- Low self-reporting of symptoms of STIs supports routine screening/treatment where possible.
- Studies to characterize pathogenesis of non-specific inflammation leading to development of interventions to reduce HIV transmission are warranted.

Background

In seeking an explanation for differential HIV transmission probabilities regionally and per sex act, genital ulceration and inflammation (GUI) emerged as important transmission co-factors in the late 1980s and early 1990s.[1–3] Decades of observational research implicate several inflammatory sexually transmitted infections (STIs) including gonorrhea, chlamydia, trichomonas and ulcerative STIs as well as syphilis and herpes simplex virus type 2 (HSV-2). Reviews[4,5] indicate that few studies consider the effect of GUIs in both sexual partners on HIV risk, or address non-STI inflammatory abnormalities such as candida, bacterial vaginosis (BV), genital discharge or inflammation more broadly.[6–9] Inflammatory processes can recruit HIV-infected or target cells to the genitalia, thereby increasing risks of both transmission and acquisition. Studies of inflammatory markers confirm that cells are associated with high viral loads (VL) in cervico-vaginal fluids,[10] and cytokine markers[11–14] and innate antimicrobial responses[15,16]

are linked to HIV acquisition. Causes of these inflammatory processes warrant closer investigation.

Though antiretroviral treatment (ART) reduces HIV-1 transmission,[17] it does not alter genital cytokine levels in HIV+ women,[18] and adherence and retention are poor in Zambia.[19–22] Low-cost strategies that reduce HIV risk independently of ART remain imperative. Building on what is known about the impact of inflammation and ulceration in heterosexual HIV transmission, we quantify the risk and population-attributable fraction (PAF) associated with a wide range of both sexually transmitted and more common non-sexually transmitted GUIs among ART-naïve Zambian serodiscordant couples over 18 years of follow-up.

Methods

Ethics

This study was approved by the Office for Human Research Protections-registered Institutional Review

Boards at Emory University and the University of Zambia. Joint written informed consent was obtained from all participating couples.

Study participants and staff

Heterosexual HIV serodiscordant couples (M+F- or F+M-) were recruited from couples' voluntary HIV counselling and testing (CVCT) in Lusaka, Zambia.[23,24] Enrolment was continuous in the open cohort through 1994–2012 and follow-up continued quarterly. Risk reduction counselling was provided at enrolment and at every visit. Free outpatient health care was provided by Zambian registered nurses, clinical officers or general medicine physicians, and specialists in internal medicine, obstetrics and gynaecology, and laboratory diagnostics. Clinical and laboratory staff received training in good clinical practices and standard operating procedures, including quality assurance and control. Couples were censored if either partner died or was lost to follow-up, the couple separated or the HIV+ partner initiated ART. Criteria for ART changed over time after it became available in government clinics, based on evolving World health organization (WHO) guidelines (CD4 < 200 cells/ml pre-2006, < 350 cells/ml post-2006).[25] HIV+ participants were referred for ART; those confirmed to have initiated ART were discharged from the cohort to reduce possible confusion from enrolment in multiple programmes.

Longitudinal data collection

Procedures changed over time as a function of available resources and study priorities. Over 1994-2002, both partners were seen quarterly and had routine genital examinations. Beginning in 2003, physical and genital examinations were performed at baseline, annually and when symptoms were reported. Plasma banking for VL testing began in 1999, and p24 antigen enzyme-linked immunosorbent assay (ELISA) screening began in 2003. Over 2007-08, HIV partners were seen at months 0,1,2 and3, and quarterly at which time sexual exposures were assessed by self-report or biological markers of condomless sex.[26] HIV partners with one or more exposures received monthly HIV testing until the next quarterly visit, at which time the assessment was repeated. Over 2008-12, all HIV partners were tested for HIV monthly. When HSV-2 ELISA was available in 2005, these tests were run on banked and new baseline samples. Due to funding constraints, follow-up was truncated at 36 months (2010) and 24 months (2011).

Exposures of interest

Exposures measured at baseline and follow-up visits included: self-reported genital ulcer, urethral or vaginal discharge, dysuria or dyspareunia; genital examination findings including external or internal genital inflammation (redness, swelling, exudate, discharge, irritation or tenderness) or genital or perianal ulceration (including erosion or friability); and laboratory studies including rapid plasma regain (RPR; BD Macro-Vue[TM], Becton-Dickinson Europe, with *T*reponema pallidum haemagglutination assays (TPHAs) confirmation when available[27]); vaginal wet mount for detection of trichomonas, clue cells (BV) and candida, including potassium hydroxide (KOH) preparations for the whiff test (BV).[28,29] Gonorrhea culture and Gram staining both routinely and in the presence of endocervical pus was piloted, but very few positive results were obtained. These expensive and time-consuming procedures were discontinued and diagnosis was based on endocervical or urethral discharge.

Diagnosis and treatment were based on the best available information including self-report, physical examination and laboratory results. Incident positive RPR prompted treatment of both partners for syphilis,[27] and self-report or clinical diagnosis of RPR-negative ulcer was treated based on clinical presentation and HSV-2 serology. Urethral discharge or dysuria in men, or endocervical discharge were detected, both partners were treated for gonorrhea and chlamydia. Both partners were treated when trichomonas was detected on vaginal wet mount, and women with candida or BV were treated given symptoms. Unilateral inguinal adenopathy (UIA) and bilateral inguinal adenopathy (BIA) were not treated.

Composite variables

For each 3-monthly interval, composite variables were created. The genital inflammation composite had two mutually exclusive levels: inflammatory STIs (clinical or laboratory diagnosis or treatment of gonorrhea, chlamydia or trichomonas); or non-inflammatory STIs (reported discharge, dysuria, dyspareunia; observed discharge or inflammation of external or internal genitalia; and/or laboratory diagnosis of candida or BV) with no indication of an inflammatory STI. The composite for genital ulcer included observed or reported ulcers and/or incident positive RPR.

Other covariates

We measured: baseline age, years cohabiting, income, literacy, current pregnancy and number of previous

pregnancies, clinical and laboratory stage as developed by our group for Kigali and modified for Zambia[30,31] and VL of HIV+ partners;[32] male circumcision (MC); HSV-2 serological positivity; STI and number of sexual partners in the past year; and contraceptive method. Time-varying measures of unprotected sex with the study partner, sperm present on a vaginal swab wet mount, contraceptive use and pregnancy were measured.

Outcome of interest

The outcome was time-to-incident HIV infection genetically linked to the study partner. HIV testing of HIV partners was conducted at 1- to 3-monthly intervals using rapid serological tests.[33] When possible, plasma from the last antibody-negative sample was tested by p24 ELISA and RNA polymerase chain reaction (PCR). To determine if infections were linked or acquired outside the partnership, conserved nucleotide sequences (gag, gp120, gp41, long terminal repeat regions) from both partners were PCR-amplified, and pairwise genetic distances were calculated for these sequenced regions.[34,35] Date of infection was defined based on available data, as the minimum of: the midpoint between the last negative and first positive antibody date (only eight seroconverters had more than 6 months between last negative and first positive visits); 2 weeks before first antigen-positive test date; or 2 weeks before first VL-positive/antibody-negative test date.

Data analysis

Analyses were conducted with SASv9.4 (SAS Institute, Cary, NC). Couples experiencing unlinked seroconversion were excluded from analysis.

HIV incidence

HIV incidence rates (the number of incident infections per couple-year (CY) of follow-up from enrolment until either the outcome occurred or the couple were censored) were calculated by months since enrolment, to explore cohort effects, and differences were evaluated by log-rank tests for linear trend. Months since enrolment were also dichotomized (months > 0-3 versus > 3), and differences evaluated by mid-*P* exact tests to compare incidence prior to joint testing and counselling (months > 0-3, reflecting transmissions occurring in the seroconversion window before CVCT) compared with rates for > 3 months of follow-up.

Exposures

Exposures are described stratified by HIV transmission status using counts and percentages (categorical variables), means and standard deviations (normal continuous

variables) and medians and interquartile ranges (non-normal continuous variables). Bivariable associations were evaluated via unadjusted Cox models. Crude hazard ratios (HRs), 95% confidence intervals (CIs), and *P*-values are reported. We also calculated the proportion of patients with STI diagnoses that reported symptoms before diagnosis, for 1994-2002.

Multivariable models

Multivariable Cox models accounting for repeated observations evaluated predictors of time-to-HIV infection. Confounding was assessed using a data-based criteria method to identify variables that changed the point estimate of any exposure of interest by +/-10%. Candidate variables were evaluated for multi-collinearity (condition indices > 30, variance decomposition proportions > 0.5); collinear variables with weaker associations with the outcome were removed. The proportional hazards assumption was confirmed for time-independent covariates. We explored for potential interaction by: cohabitation length (< 1 year versus ≥ 1 year); circumcision and smegma; and circumcision, smegma and BIA. A 'generalized' R-squared statistic (with > 60% thought to be meaningful) was calculated for each model as:[36]

$$R^2 = 1 - e^{-\left(\frac{LRT}{n}\right)}$$

where LRT (likelihood ratio test) is the difference between the -2log likelihood for the null model with no covariates and the fitted model, and n is the number of observations. Adjusted hazard ratios (aHRs) are reported.

Population-attributable fractions

PAFs were calculated for genital inflammation and ulceration composites using standard formulae[37–39] with aHRs as the measure of relative risk:[40–42]

$$PAF = \left[\frac{(aHR - 1)}{aHR}\right] * p$$

where p is the proportion of cases exposed. Confidence intervals around PAFs were calculated.[43,44]

Sub-analyses

For the data collected over 1994-2002, we explored interactions between circumcision, foreskin smegma and BIA in men.

Sensitivity analyses

To explore the possibility of GUI exposure misclassification, we built models assuming a random 15% of exposures preceding seroconversion were incorrectly classified as

negative, and another model assuming 15% of GUI exposures preceding seroconversion were incorrectly classified as positive. We also constructed a model after multiple imputation, carried out using Markov Chain Monte Carlo methods which assume that the variables have a joint multivariate normal distribution. Simulation studies indicate that this common imputation method typically leads to robust estimates regardless of true normality.[45,46]

Results

Follow-up and transmission

A total of 1348 M+F- couples were followed for a median of 430 (interquartile range = 767) days; 207 linked transmissions occurred in women over 2756 CY (7.5/100 CY; 95% CI: 6.5-8.6). Follow-up among 1601 F+M- couples was a median of 448 (interquartile range = 730) days; 171 linked transmissions occurred in men over 3216 CY (5.3/100 CY; 95% CI: 4.5-6.2). During this time, 45 unlinked infections occurred in women and 55 in men; these couples were excluded from analysis. Among study couples, 57% had at least 1 year of follow-up, 35% had $\geq$ 2 years and 22% had $\geq$ 3 years.

HIV incidence

Incidence in women decreased between > 0-3 months (13.0/100 CY; 95% CI: 9.4-17.5) versus > 3 months of follow-up (6.7/100 CY; 95% CI: 5.7-7.8) ($P < 0.001$, Figure 1A). This was also true for men: > 0-3 months (8.7/100 CY; 95% CI: 6.0-12.2) versus > 3 months (4.8/100 CY; 95% CI: 4.0-5.7) ($P < 0.001$, Figure 1B). Rates of transmission from M+F- were consistently higher than for F+M-. Follow-up time was predictive in unadjusted and adjusted models (> 0-3 months versus > 3 months, $P < 0.001$).

Baseline exposures

Exposures associated with transmission included lower men's and women's ages, fewer years cohabiting, fewer previous pregnancies and increasing baseline VL of the HIV+ partner (Table 1). Among M+F- couples, the woman's illiteracy and pregnancy at baseline were associated with transmission. Among F+M- couples, the man being uncircumcised was associated with transmission.

Time-varying exposures

Time-varying exposures associated with male-to-female transmission included non-STI inflammation in women and STI inflammation, genital ulceration and BIA in both partners (Table 2). Female-to-male transmission was associated with non-STI genital inflammation and BIA in both partners and genital ulceration in men.

Multivariable models

Collinear variables included men's and women's ages, ages and years cohabiting and age and number of previous pregnancies (Tables 3 and 4). Women's age was retained. There was no interaction by cohabitation length. Genital ulcer of HIV+ women was not included in multivariable models because of lower statistical significance and magnitude of the crude point estimate.

Incident HIV among women was associated with the woman's non-STI genital inflammation (aHR = 1.6; PAF = 8%), BIA (aHR = 2.3; PAF = 8%) and genital ulceration (aHR = 2.1; PAF = 7%), and the man's STI genital inflammation (aHR = 3.3; PAF = 5%), BIA (aHR = 3.4; PAF = 33%) and genital ulceration (aHR = 1.5; PAF = 9%). The 'generalized' R-squared statistic was 63%.
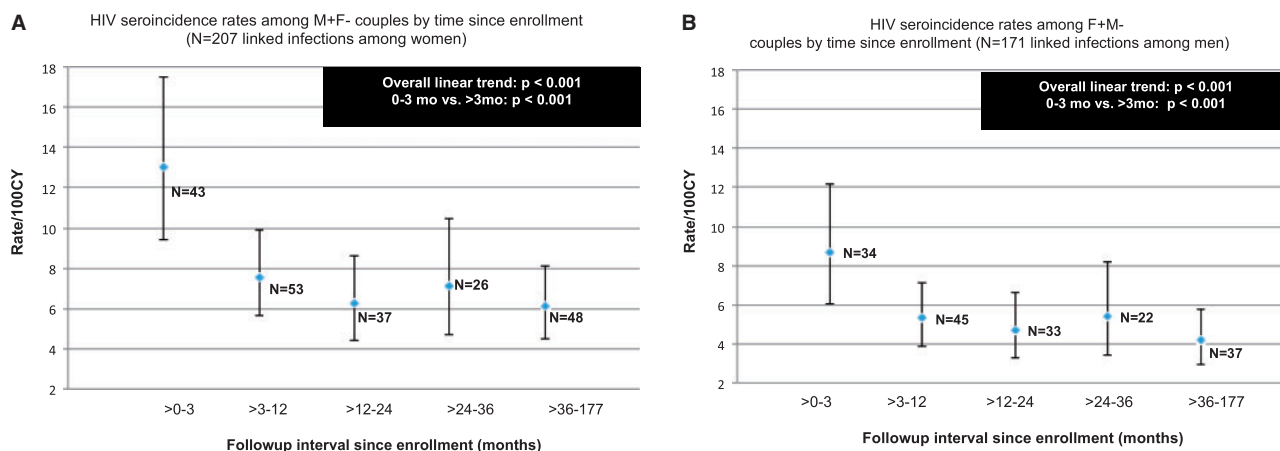

Figure 1. HIV seroincidence rates per 100 couple years (CY) and 95% confidence interval bars among Zambian women and men in HIV discordant relationships, Ns indicate number of seroconversions occurring in each time interval.

Table 1. Descriptive analyses of baseline covariates by seroconversion outcomes among Zambian women and men in HIV discordant relationships

	M+F- couples						F+M- couples					
	Non-seroconverters (N = 1141)	Linked transmissions (N = 207)	HR	95% CI		P-value	Non-seroconverters (N = 1430)	Linked transmissions (N = 171)	HR	95% CI		P-value
	N (%, SD)	N (%, SD)					N (%, SD)	N (%, SD)				
Age of man (years)[a]	35.5 (7.7)	33.5 (7.5)	0.97	0.95	0.99	< 0.001	35.4 (8.5)	33.1 (8.0)	0.97	0.95	0.99	0.001
Age of woman (years)[a]	28.8 (7.0)	26.3 (6.3)	0.96	0.94	0.98	< 0.001	29.0 (6.8)	27.0 (6.2)	0.97	0.94	0.99	0.007
Years cohabiting[a]	8.3 (6.7)	6.7 (5.7)	0.96	0.94	0.99	0.002	6.0 (6.0)	4.7 (5.0)	0.95	0.92	0.98	0.002
Monthly household income (USD)[b]	62.1 (85.6)	49.1 (61.6)	1.00	0.997	1.001	0.35	64.2 (84.6)	50.0 (55.7)	1.00	0.997	1.001	0.26
Woman reads Nyanja												
Yes, easily	272 (24)	35 (17)	ref				379 (27)	36 (22)	ref			
With difficulty/no	857 (76)	167 (83)	1.45	1.01	2.09	0.05	1037 (73)	131 (78)	1.19	0.83	1.73	0.35
# previous pregnancie[a]	3.7 (2.5)	3.1 (2.1)	0.90	0.85	0.96	0.001	3.2 (2.2)	3.0 (2.2)	0.90	0.83	0.98	0.02
HIV stage of positive partner												
Stage I	294 (26)	49 (24)	1.06	0.73	1.52	0.77	600 (42)	64 (37)	0.92	0.63	1.34	0.66
Stage II	369 (33)	81 (39)	1.04	0.76	1.43	0.80	398 (28)	55 (32)	1.00	0.68	1.46	0.99
Stage III-IV	467 (41)	76 (37)	ref				420 (30)	52 (30)	ref			
VL of positive partner ($\log_{10}$ copies/ml)[a]	4.6 (1.0)	5.1 (0.7)	1.66	1.36	2.04	< 0.001	4.3 (0.9)	4.8 (0.7)	1.45	1.23	1.71	< 0.001
Circumcised man[c]												
Yes	89 (8)	15 (7)	ref				267 (19)	12 (7)	ref			
No	1039 (92)	191 (93)	1.22	0.72	2.06	0.47	1160 (81)	159 (93)	2.69	1.49	4.85	0.001
RPR status of man												
Positive	83 (7)	22 (11)	1.15	0.74	1.81	0.39	95 (7)	22 (13)	1.26	0.80	1.97	0.32
Negative	1057 (93)	185 (89)	ref				1333 (93)	149 (87)	ref			
RPR status of woman												
Positive	74 (6)	16 (8)	0.93	0.56	1.56	0.79	113 (8)	24 (14)	1.14	0.74	1.76	0.56
Negative	1066 (94)	191 (92)	ref				1315 (92)	147 (86)	ref			

Variables not associated with the outcome (not tabled): contraceptive method at baseline, male literacy, past year history of sexually transmitted infections, number of sexual partners in the last year, HSV-2 serology status of woman or man. *P*-values are two-tailed from crude (unadjusted) Cox models. Viral load collected beginning in 1999. Counts may not sum to total intervals due to missing data.

USD, United States dollar; ref, reference value; SD, standard deviation.

[a]Indicates a continuous variable, mean and standard deviation reported and HRs are estimated per unit increase.

[b]Indicates a non-normally distributed continuous variable, median and interquartile range reported.

[c]Circumcised at baseline or ever during follow-up.

Table 2. Descriptive analyses of time-varying covariates by HIV seroconversion outcomes among Zambian women and men in HIV discordant relationships

Exposures of interest	M+F− couples						F+M− couples					
	Non-seroconverter (N = 13547 intervals) N (%, SD)	Linked transmission (N = 207 intervals) N (%, SD)	HR	95%CI		P-value	Non-seroconverter (N = 13,237 intervals) N (%, SD)	Linked transmission (N = 171 intervals) N (%, SD)	HR	95%CI		P-value
Genital inflammation of woman												
STI	500 (4)	20 (10)	2.48	1.54	4.01	< 0.001	851 (6)	15 (9)	1.61	0.92	2.82	0.10
Non-STI	1282 (10)	44 (21)	1.86	1.25	2.77	< 0.01	1459 (11)	45 (26)	3.11	1.97	4.90	< 0.001
No	11675 (87)	144 (69)	ref				10927 (83)	111 (65)	ref			
BIA of woman												
Yes	299 (2)	30 (14)	4.43	2.88	6.81	< 0.001	1057 (8)	38 (22)	2.84	1.82	4.43	< 0.001
No	13158 (98)	177 (86)	ref				12180 (92)	133 (78)	ref			
Genital ulcer of woman												
Yes	573 (4)	29 (14)	3.00	1.97	4.56	< 0.001	1118 (8)	26 (15)	1.51	0.97	2.36	0.07
No	12884 (96)	178 (86)	ref				12119 (92)	145 (85)	ref			
Genital inflammation of man												
STI	189 (1)	14 (7)	4.01	2.31	6.98	< 0.001	145 (1)	2 (1)	1.02	0.25	4.16	0.98
Non-STI	111 (1)	5 (3)	2.03	0.82	5.05	0.127	188 (1)	10 (6)	2.68	1.36	5.26	< 0.01
No	13157 (98)	188 (91)	ref				12904 (97)	159 (93)	ref			
BIA of man												
Yes	2451 (18)	98 (47)	4.49	3.07	6.58	< 0.001	859 (6)	49 (29)	6.29	4.05	9.75	< 0.001
No	11006 (82)	109 (53)	ref				12378 (94)	122 (71)	ref			
Genital ulcer of man												
Yes	1824 (14)	52 (25)	1.74	1.26	2.41	0.001	719 (5)	37 (22)	5.02	3.43	7.35	< 0.001
No	11633 (86)	155 (75)	ref				12518 (95)	134 (78)	ref			
Sexual behaviour and family planning characteristics												
No. unprotected sex acts with study partner since last visit[a]	2.4 (8.5)	3.7 (10.3)	1.00	0.99	1.01	0.89	3.9 (12.4)	9.9 (21.8)	1.02	1.01	1.02	< 0.001
Any unprotected sex with study partner since last visit												
Yes	4087 (30)	87 (42)	1.39	1.04	1.84	0.02	4646 (36)	100 (58)	2.39	1.75	3.25	< 0.001
No	9433 (70)	119 (58)	ref				8409 (64)	71 (42)	ref			
Sperm present on vaginal swab wet mount												
Yes	831 (6)	25 (13)	1.52	0.98	2.37	0.06	811 (6)	25 (15)	2.03	1.28	3.21	< 0.01
No	12685 (94)	183 (87)	ref				12420 (94)	147 (85)	ref			

(continued)

Table 2. Continued

Exposures of interest	M+F- couples						F+M- couples					
	Non-sero-converter (N = 13547 intervals) N (%, SD)	Linked transmission (N = 207 intervals) N (%, SD)	HR	95%CI		P-value	Non-sero-converter (N = 13,237 intervals) N (%, SD)	Linked transmission (N = 171 intervals) N (%, SD)	HR	95%CI		P-value
Current pregnancy												
Yes	1130 (9)	27 (14)	1.41	0.94	2.12	0.097	819 (7)	26 (16)	2.27	1.49	3.46	< 0.001
No	11168 (91)	171 (86)	ref				10575 (93)	135 (84)	ref			
Incident pregnancy												
Yes	487 (4)	16 (8)	1.40	0.84	2.34	0.20	435 (4)	11 (7)	1.56	0.84	2.88	0.16
No	11253 (96)	185 (92)	ref				10838 (96)	150 (93)	ref			
Associations with foreskin smegma (pre-2002 only)												
Circumcision status by smegma												
Circumcised	383 (9)	6 (6)	ref				737 (20)	7 (10)	ref			
Uncircumcised and no smegma	3356 (82)	78 (78)	1.49	0.65	3.41	0.35	2580 (71)	49 (71)	2.06	0.93	4.57	0.08
Uncircumcised and smegma	367 (9)	16 (16)	2.82	1.10	7.24	0.03	323 (9)	13 (19)	3.73	1.47	9.45	0.01
Circumcision status by man's BIA												
Circumcised												
BIA of man: Yes	224 (5)	2 (2)	0.42	0.08	2.32	0.32	125 (3)	1 (1)	0.91	0.11	7.68	0.93
BIA of man: No	159 (4)	4 (4)	ref				612 (17)	6 (9)	ref			
Uncircumcised and no smegma												
BIA of man: Yes	1773 (43)	51 (51)	1.25	0.44	3.57	0.67	542 (15)	27 (39)	5.28	2.12	13.15	< 0.001
BIA of man: No	1583 (39)	27 (27)	0.75	0.26	2.17	0.60	2038 (56)	22 (32)	1.17	0.47	2.90	0.74
Uncircumcised and smegma												
BIA of man: Yes	226 (6)	12 (12)	2.32	0.73	7.45	0.16	114 (3)	6 (9)	5.50	1.72	17.58	< 0.01
BIA of man: No	141 (3)	4 (4)	1.34	0.33	5.48	0.68	209 (6)	7 (10)	3.22	1.07	9.74	0.04

Foreskin smegma recorded from 1994 to 2002. *P*-values are two-tailed from Cox models. Variables not associated with the outcome (not tabled): time-varying contraceptive methods, unilateral inguinal adenopathy.

Counts may not sum to total intervals due to missing data.

[a]Indicates a continuous variable, mean and standard deviation reported.

Table 3. Multivariable model of predictors of time to HIV transmission among Zambian women and men in HIV discordant relationships

Primary model[a,b]	M+F- couples				F+M- couples			
Exposures of interest	aHR	95% CI		*P*-value	aHR	95% CI		*P*-value
Genital inflammation of woman								
STI	1.36	0.76	2.43	0.30	1.01	0.50	2.05	0.97
Non-STI	1.55	1.01	2.38	0.04	1.92	1.15	3.22	0.01
No	ref				ref			
BIA of woman								
Yes	2.33	1.43	3.81	< 0.01	2.76	1.69	4.51	< 0.001
No	ref				ref			
Genital ulcer of woman								
Yes	2.08	1.27	3.41	< 0.01				
No	ref							
Genital inflammation of man								
STI	3.33	1.79	6.17	< 0.01	e			
Non-STI	1.18	0.43	3.21	0.75	1.19	0.51	2.77	0.69
No	ref				ref			
BIA of man								
Yes	3.35	2.24	5.03	< 0.001	4.11	2.52	6.72	< 0.001
No	ref				ref			
Genital ulcer of man								
Yes	1.49	1.05	2.11	0.03	3.44	2.20	5.38	< 0.001
No	ref				ref			
Baseline and time-varying variables								
Age of woman (per year increase)	0.98	0.95	1.00	0.04	0.99	0.96	1.02	0.42
Woman reads Nyanja								
Yes, easily	ref							
With difficulty/not at all	1.22	0.82	1.80	0.32				
Circumcised man[c]								
Yes					ref			
No					2.10	1.12	3.95	0.02
VL of positive partner (per $\log_{10}$ copies/ml increase)	1.68	1.35	2.08	< 0.001	1.80	1.44	2.23	< 0.001
Any self-reported unprotected sex with study partner since last visit								
Yes	1.38	1.01	1.88	0.04	1.91	1.33	2.75	< 0.01
No	ref				ref			
Sperm present on vaginal swab wet mount								
Yes	1.41	0.88	2.26	0.15	1.73	1.03	2.91	0.04
No	ref				ref			
Current pregnancy								
Yes					1.35	0.79	2.31	0.27
No					ref			
Interval since enrolment								
0–3 months	4.30	2.75	6.72	< 0.001	5.07	2.93	8.78	< 0.001
< 3 months	ref				ref			
Pre-2002 model 1[d]								
Circumcision and smegma status								
Circumcised	ref				ref			
Uncircumcised and no smegma	1.68	0.93	3.04	0.08	4.50	2.36	8.59	< 0.001
Uncircumcised and smegma	3.16	1.54	6.46	< 0.01	8.59	3.72	19.86	< 0.001
Pre-2002 model 2[d]								
Circumcision status and smegma by man's BIA								
Circumcised								
BIA of man: Yes					0.36	0.06	1.99	0.24
BIA of man: No					ref			

Table 3. Continued

Primary model[a,b]	M+F- couples			F+M- couples			
Exposures of interest	aHR	95% CI	*P*-value	aHR	95% CI		*P*-value
Uncircumcised and no smegma							
BIA of man: Yes				4.95	2.43	10.10	< 0.001
BIA of man: No				0.85	0.31	2.34	0.75
Uncircumcised and smegma							
BIA of man: Yes				13.03	4.97	34.17	< 0.0001
BIA of man: No					e		

Foreskin smegma recorded from 1994-2002. *P*-values are two-tailed from Cox models. Adjusted point estimate for genital ulcer of woman in F+M- primary model (not included in final models): aHR = 0.94, 95% CI:0.55-1.62 (*P* = 0.84).

[a]'Generalized' R-squared = 0.63 for M+F- couples (primary model).
[b]'Generalized' R-squared = 0.80 for F+M- couples (primary model).
[c]Circumcised at baseline or ever during follow-up.
[d]Controlling for the primary model variables.
[e]Small sample size; a measure of association could not be estimated.

Infection among men was associated with the woman's non-STI genital inflammation (aHR = 1.9; PAF = 13%) and BIA (aHR = 2.8; PAF = 14%), and the man's BIA (aHR = 4.1; PAF = 22%) and genital ulceration (aHR = 3.4; PAF = 15%). The 'generalized'R-squared statistic was 80%.

In M+F- couples, 84% of trichomonas were detected without self-reported discharge; 78% (men) and 92% (women) of incident positive RPR results were detected with no self-reported genital ulcers; 32% (men) and 71% (women) of incident RPR-negative ulcers were detected without self- reported ulcer; and 28% (men) and 98% (women) of cases of gonorrhea and chlamydia were diagnosed with no self-reported symptoms.

In F+M- couples, 86% of trichomonas were detected without self-reported discharge; 90% (men and women) of incident positive RPR results were detected with no self-reported genital ulcers; 20% (men) and 65% (women) of incident RPR-negative ulcers were detected without self-reported ulcer; and 20% (men) and 92% (women) of cases of gonorrhea and chlamydia were diagnosed with no self-reported symptoms.

1994–2002 sub-analyses

In M+F- couples, being uncircumcised with foreskin smegma was associated with seroconversion (aHR = 3.2) relative to being circumcised. In F+M- couples, uncircumcised men with BIA had an increased hazard of seroconversion (aHR = 5.0 without smegma, aHR = 13.0 with smegma) relative to being circumcised (Table 3).

Sensitivity analyses

If 15% of GUI exposures before seroconversion were misclassified as false-negative, GUI exposures are more hazardous than in primary analyses (Appendix 1, available as Supplementary data at *IJE* online), whereas if 15% of these GUI exposures were misclassified as false-positive, estimates were tempered toward the null (Appendix 2, available as Supplementary data at *IJE* online). For every variable in primary analyses, 87% of M+F- and F+M- cases were complete, and multiple imputation results were similar to primary analyses, with slightly more hazardous point estimates (Appendix 3, available as Supplementary data at *IJE* online).

Discussion

Our findings confirm the role of ulcerative STIs and highlight the contribution of non-ulcerative STIs and non-specific genital inflammation in HIV transmission risk in both donor and recipient in both M+F- and F+M- couples. The high PAF contributed by BIA merits further investigation. Genital practices may be a contributing factor and should be investigated.

As in other studies,[40,47] ulcerative STIs contributed to a substantial PAF of transmission in both men and women donors and women seroconverters. Most incident ulcers diagnoses were asymptomatic and detected during routine physical examinations or screening. Syphilis is common in Zambia,[48] and routine RPR screening is inexpensive and does not require sophisticated laboratory equipment or electricity, and penicillin treatment is inexpensive with no documented resistance.[27]

We did not find prevalent HSV-2 antibody associated with HIV transmission in our analysis, but this is not surprising as only a minority of HSV-2 antibody-positive persons developed detectable ulcers. When RPR-negative ulcers were detected, they were generally assumed to be herpetic and

Table 4. Attributable fraction in the population for exposures of interest significant in multivariable models

Primary models	M+F-			F+M-		
	AFp	95% CI		AFp	95% CI	
Genital inflammation of woman: Non-STI	8%	3.71	16.73	13%	7.48	21.85
BIA of woman	8%	5.94	11.75	14%	11.24	17.93
Genital ulcer of woman	7%	4.79	11.42			
Genital inflammation of man: STI	5%	3.77	6.02			
BIA of man	33%	30.19	36.58	22%	18.56	25.36
Genital ulcer of man	8%	4.24	17.17	15%	13.88	16.97
Pre-2002 model 1						
Uncircumcised and no smegma				55%	46.17	66.12
Uncircumcised and smegma	11%	8.12	14.86	17%	14.89	18.63
Pre-2002 model 2						
Uncircumcised and no smegma and BIA of man				31%	26.18	37.29
Uncircumcised and smegma and BIA of man				8%	7.17	9.01

Foreskin smegma recorded from 1994-2002.

treated with acyclovir when it became available. Though Phase III clinical trials of acyclovir for HSV-2 antibody-positivity have not decreased HIV risk,[49–51] acyclovir treatment of visible ulcers in RPR- patients is still advisable. Men and women could be encouraged to examine external genitalia and seek treatment for visible ulcers.

Inflammatory STIs in HIV+ men were risk factors for women's HIV acquisition. As with ulcers, many inflammatory STIs were asymptomatic – particularly in women who cannot inspect their internal genitalia – and were diagnosed during routine physical examination or screening. Many countries, including Zambia, rely on syndromic STI detection, an approach associated with reduced HIV incidence in neighbouring Tanzania.[52] In contrast, mass population treatment of presumptive STI has not resulted in reduced HIV incidence in most settings.[53] New diagnostics for gonorrhea, chlamydia and trichomonas may be useful in screening asymptomatic disease.[54]

Inflammation not due to STI included reported symptoms, visible abnormalities on genital examination, and/or diagnosed candida or BV. Non-STI inflammation in women was associated both transmission and acquisition. BIA contributed a surprisingly high PAF to transmission in both HIV+ and HIV- men and women. Lymphadenopathy is non-specific and typically as reaction to infection; HIV-associated lymphadenopathy can last 2-3 months.[55] To develop testable interventions to reduce discharge and non-specific inflammation without compromising genital integrity, an understanding of current genital practices is needed. In Zambia, as in many sub-Saharan African countries, women engage in intra-vaginal practices in response to discharge, genital disturbance, or for cultural reasons, which can lead to inflammatory processes, abrasion and increased STI risk.[56–58] Additionally, highly diverse non-lactobacillus-dominated bacterial communities have been associated with inflammation facilitating HIV transmission,[59–61] and a better understanding of the microbiome is needed.[62–64] Education ideally before initiation of sexual activity, to increase recognition and management of abnormal vaginal discharges, odours or discomfort, is indicated.

As expected, lack of circumcision was independently predictive of seroconversion in F+M- couples. BIA was not associated with increased risk of seroconversion in circumcised HIV- men. Being uncircumcised with smegma was associated with risk of both transmitting and acquiring HIV. The presence of smegma increased the risk associated with BIA. We support the scale-up targeted voluntary MC for HIV- men with HIV+ partners. In settings like Zambia where circumcision is not a tradition and MC is low, research on men's genital hygiene practices is needed. Foreskin smegma has been associated with inguinal adenopathy in Rwandan men,[65] and a study of Ugandan men found foreskin inflammation to be associated with higher VL and smegma.[66]

We observed a decrease in infections at the time of enrolment versus subsequent months, paralleling the reported onset of condom use after CVCT. These data suggest GUIs contribute a substantial proportion of new HIV infections in ART-naïve serodiscordant couples after CVCT. In Zambia, virological failure, transmitted resistance[21,67,68] and GUI-associated transmission of multiple HIV-variants[66,69,70] have been noted.

Limitations

We previously published analyses of possible selective enrolment and retention biases which may limit generalizability.[71] A possible selection bias for healthier index partners and

information bias in self-reported exposure variables could be differential by risk, biasing our results in an unknown direction. It is possible that couples with GUIs were more likely to have follow-up visits, though baseline STIs were not associated with retention.[71] Though we attempt to control for unprotected sex to assess the independent effect of genital inflammation, uncontrolled confounding could make the latter a proxy measure of unprotected sex.

Conclusions

Our findings confirm the role of STIs and highlight the contribution of non-specific genital inflammation to HIV transmission and acquisition risk in both HIV+ and HIV- men and women. A multipronged approach will be needed that maximizes detection, management and prevention. More research to develop low-cost, sustainable interventions to reduce genital co-factors for HIV transmission is warranted.

Supplementary Data

Supplementary data are available at *IJE* online.

Author Contributions

K.M.W. contributed to the analysis and interpretation of data, drafted the article and revised it critically for important intellectual content and gave final approval of the version to be published. B.V. contributed to the conception and design of the study, revised the article critically for important intellectual content and gave final approval of the version to be published. S.L. contributed to the conception and design of the study, revised the article critically for important intellectual content and gave final approval of the version to be published. R.C. contributed to the conception and design of the study, revised the article critically for important intellectual content and gave final approval of the version to be published. W.K. contributed to the conception and design of the study, revised the article critically for important intellectual content and gave final approval of the version to be published. L.H. contributed to the analysis and interpretation of data, drafted the article and revised it critically for important intellectual content and gave final approval of the version to be published. N.H.K. contributed to the analysis and interpretation of data, revised the article critically for important intellectual content and gave final approval of the version to be published. I.B. contributed to the analysis and interpretation of data, revised the article critically for important intellectual content and gave final approval of the version to be published. C.V. contributed to the conception and design of the study, revised the article critically for important intellectual content and gave final approval of the version to be published. L.M. contributed to the conception and design of the study, revised the article critically for important intellectual content and gave final approval of the version to be published. E.C. contributed to the conception and design of the study, revised the article critically for important intellectual content and gave final approval of the version to be published. J.M. contributed to the conception and design of the study, revised the article critically for important intellectual content and gave final approval of the version to be published. A.T. contributed to the study conception and design, revised the article critically for important intellectual content and gave final approval of the version to be published. S.A. contributed to the study design and conception, contributed to the analysis and interpretation of data, revised the article critically for important intellectual content and gave final approval of the version to be published.

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References

1. Laga M, Manoka A, Kivuvu M a. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. *AIDS* 1993;**7**:95–102.
2. Cameron DW, Simonsen JN, D'Costa LJ *et al.* Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men. *Lancet* 1989;**2**:403–07.
3. Plummer FA, Simonsen JN, Cameron DW *et al.* Cofactors in male-female sexual transmission of human immunodeficiency virus type 1. *J Infect Dis* 1991;**163**:233–39.
4. Mayer KH, Venkatesh KK. Interactions of HIV, other sexually transmitted diseases, and genital tract inflammation facilitating local pathogen transmission and acquisition. *Am J Reprod Immunol* 2011;**65**:308–16.
5. Ward H, Ronn M. Contribution of sexually transmitted infections to the sexual transmission of HIV. *Curr Opin HIV AIDS* 2010;**5**:305–10.
6. van de Wijgert J, Morrison C, Salata R, Padian N. Is vaginal washing associated with increased risk of HIV-1 acquisition? *AIDS* 2006;**20**:1347–48.

7. van de Wijgert JH, Morrison CS, Brown J *et al*. Disentangling contributions of reproductive tract infections to HIV acquisition in African Women. *Sex Transm Dis* 2009;**36**:357–64.

8. Cohen CR, Lingappa JR, Baeten JM *et al*. Bacterial vaginosis associated with increased risk of female-to-male HIV-1 transmission: a prospective cohort analysis among African couples. *PLoS Med* 2012;**9**:e1001251.

9. Masese L, Baeten JM, Richardson BA *et al*. Changes in the contribution of genital tract infections to HIV acquisition among Kenyan high-risk women from 1993 to 2012. *AIDS* 2015;**29**:1077–85.

10. Homans J, Christensen S, Stiller T *et al*. Permissive and protective factors associated with presence, level, and longitudinal pattern of cervicovaginal HIV shedding. *J Acquir Immune Defic Syndr* 2012;**60**:99–110.

11. Passmore JA, Jaspan HB, Masson L. Genital inflammation, immune activation and risk of sexual HIV acquisition. *Curr Opin HIV AIDS* 2016;**11**:156–62.

12. Masson L, Passmore JA, Liebenberg LJ *et al*. Genital inflammation and the risk of HIV acquisition in women. *Clin Infect Dis* 2015;**61**:260–09.

13. Masson L, Arnold KB, Little F *et al*. Inflammatory cytokine biomarkers to identify women with asymptomatic sexually transmitted infections and bacterial vaginosis who are at high risk of HIV infection. *Sex Transm Infect* 2016;**92**:186–93.

14. Esra RT, Olivier AJ, Passmore JA, Jaspan HB, Harryparsad R, Gray CM. Does HIV Exploit the Inflammatory Milieu of the Male Genital Tract for Successful Infection? *Front Immunol* 2016;**7**:245.

15. Pellett Madan R, Masson L, Tugetman J *et al*. Innate Antibacterial Activity in Female Genital Tract Secretions Is Associated with Increased Risk of HIV Acquisition. *AIDS Res Hum Retroviruses* 2015;**31**:1153–59.

16. Pellett Madan R, Dezzutti CS, Rabe L *et al*. Soluble Immune Mediators and Vaginal Bacteria Impact Innate Genital Mucosal Antimicrobial Activity in Young Women. *Am J Reprod Immunol* 2015;**74**:323–32.

17. Cohen MS, Chen YQ, McCauley M *et al*. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med* 2011;**365**:493–505.

18. Ondoa P, Gautam R, Rusine J *et al*. Twelve-Month Antiretroviral Therapy Suppresses Plasma and Genital Viral Loads but Fails to Alter Genital Levels of Cytokines, in a Cohort of HIV-Infected Rwandan Women. *PLoS One* 2015;**10**:e0127201.

19. Denison JA, Koole O, Tsui S *et al*. Incomplete adherence among treatment-experienced adults on antiretroviral therapy in Tanzania, Uganda and Zambia. *AIDS* 2015;**29**:361–71.

20. Koole O, Tsui S, Wabwire-Mangen F *et al*. Retention and risk factors for attrition among adults in antiretroviral treatment programmes in Tanzania, Uganda and Zambia. *Trop Med Int Health* 2014;**19**:1397–410.

21. Chi BH, Cantrell RA, Zulu I *et al*. Adherence to first-line antiretroviral therapy affects non-virologic outcomes among patients on treatment for more than 12 months in Lusaka, Zambia. *Int J Epidemiol* 2009;**38**:746–56.

22. Keiser O, Chi BH, Gsponer T *et al*. Outcomes of antiretroviral treatment in programmes with and without routine viral load monitoring in Southern Africa. *AIDS* 2011;**25**:1761–19.

23. Allen S, Karita E, Chomba E *et al*. Promotion of couples' voluntary counselling and testing for HIV through influential networks in two African capital cities. *BMC Public Health* 2007;**7**:349.

24. Wall KM, Kilembe W, Nizam A *et al*. Promotion of couples' voluntary HIV counselling and testing in Lusaka, Zambia by influence network leaders and agents. *BMJ Open* 2012;**2**. PMID: 22956641.

25. World Health Organization. *Antiretroviral Therapy for Hiv Infection in Adults and Adolescents*. Geneva: WHO, 2006.

26. Allen S, Meinzen-Derr J, Kautzman M *et al*. Sexual behavior of HIV discordant couples after HIV counseling and testing. *AIDS* 2003;**17**:733–40.

27. Dionne-Odom J, Karita E, Kilembe W *et al*. Syphilis treatment response among HIV-discordant couples in Zambia and Rwanda. *Clin Infect Dis* 2013;**56**:1829–37.

28. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983;**74**:14–22.

29. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol* 1991;**29**:297–301.

30. Lifson AR, Allen S, Wolf W *et al*. Classification of HIV infection and disease in women from Rwanda. Evaluation of the World Health Organization HIV staging system and recommended modifications. *Ann Intern Med* 1995;**122**:262–70.

31. Peters PJ, Zulu I, Kancheya NG *et al*. Modified Kigali combined staging predicts risk of mortality in HIV-infected adults in Lusaka, Zambia. *AIDS Res Hum Retroviruses* 2008;**24**:919–24.

32. 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.

33. Boeras DI, Luisi N, Karita E *et al*. Indeterminate and discrepant rapid HIV test results in couples' HIV testing and counselling centres in Africa. *J Int AIDS Soc* 2011;**14**:18.

34. Trask SA, Derdeyn CA, Fideli U *et al*. Molecular epidemiology of human immunodeficiency virus type 1 transmission in a heterosexual cohort of discordant couples in Zambia. *J Virol* 2002;**76**:397–405.

35. Eshleman SH, Hudelson SE, Bruce R *et al*. Analysis of HIV type 1 gp41 sequences in diverse HIV type 1 strains. *AIDS Res Hum Retroviruses* 2007;**23**:1593–98.

36. Allison PD. *Survival Analysis Using the SAS System: A Practical Guide*. Cary, NC: SAS Institute Inc., 1995.

37. Greenland S. Bias in methods for deriving standardized morbidity ratio and attributable fraction estimates. *Stat Med* 1984;**3**:131–41.

38. Levin ML. The occurrence of lung cancer in man. *Acta Unio Int Contra Cancrum* 1953;**9**:531–41.

39. Miettinen OS. Proportion of disease caused or prevented by a given exposure, trait or intervention. *Am J Epidemiol* 1974;**99**:325–32.

40. Ashraf S, Huque MH, Kenah E, Agboatwalla M, Luby SP. Effect of recent diarrhoeal episodes on risk of pneumonia in children under the age of 5 years in Karachi, Pakistan. *Int J Epidemiol* 2013;**42**:194–200.

41. Benichou J. A review of adjusted estimators of attributable risk. *Stat Methods Med Res* 2001;**10**:195–216.

42. Benichou J. [Biostatistics and epidemiology: measuring the risk attributable to an environmental or genetic factor.] *Comptes Rendus Biol* 2007;**330**:281–98.

43. Newcombe RG. Re: "Confidence limits made easy: interval estimation using a substitution method". *Am J Epidemiol* 1999;**149**:884–85; author reply 885-86.

44. Greenland S. Re: "Confidence limits made easy: interval estimation using a substitution method". *Am J Epidemiol* 1999;**149**:884; author reply 885-86.

45. Lee KJ, Carlin JB. Multiple imputation for missing data: fully conditional specification versus multivariate normal imputation. *Am J Epidemiol* 2010;**171**:624–32.

46. Demirtas H, Freels SA, Yucel RM. Plausibility of multivariate normality assumption when multiply imputing non-gaussian continuous outcomes: a simulation assessment. *J Stat Comput Simulation* 2008;**78**:69–84.

47. 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.

48. Dunkle KL, Stephenson R, Karita E *et al*. New heterosexually transmitted HIV infections in married or cohabiting couples in urban Zambia and Rwanda: an analysis of survey and clinical data. *Lancet* 2008;**371**:2183–91.

49. Celum C, Wald A, Hughes J *et al*. Effect of aciclovir on HIV-1 acquisition in herpes simplex virus 2 seropositive women and men who have sex with men: a randomised, double-blind, placebo-controlled trial. *Lancet* 2008;**371**:2109–19.

50. Celum C, Wald A, Lingappa JR *et al*. Acyclovir and transmission of HIV-1 from persons infected with HIV-1 and HSV-2. *N Engl J Med* 2010;**362**:427–39.

51. Watson-Jones D, Weiss HA, Rusizoka M *et al*. Effect of herpes simplex suppression on incidence of HIV among women in Tanzania. *N Engl J Med* 2008;**358**:1560–71.

52. Grosskurth H, Mosha F, Todd J *et al*. Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. *Lancet* 1995;**346**: 530–36.

53. Stillwaggon E, Sawers L. Rush to judgment: the STI-treatment trials and HIV in sub-Saharan Africa. *J Int AIDS Soc* 2015;**18**:19844.

54. Herbst de Cortina S, Bristow CC, Joseph Davey D, Klausner JD. A Systematic Review of Point of Care Testing for Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis. *Infect Dis Obstet Gynecol* 2016;**2016**:4386127.

55. Mohseni S, Shojaiefard A, Khorgami Z, Alinejad S, Ghorbani A, Ghafouri A. Peripheral lymphadenopathy: approach and diagnostic tools. *Iran J Med Sci* 2014;**39**(**Suppl 2**):158–70.

56. Alcaide ML, Chisembele M, Malupande E, Arheart K, Fischl M, Jones DL. A cross-sectional study of bacterial vaginosis, intravaginal practices and HIV genital shedding; implications for HIV transmission and women's health. *BMJ Open* 2015;**5**:e009036.

57. Alcaide ML, Cook R, Chisembele M, Malupande E, Jones DL. Determinants of intravaginal practices among HIV-infected women in Zambia using conjoint analysis. *Int J STD AIDS* 2016;**27**:453–61.

58. Alcaide ML, Chisembele M, Mumbi M, Malupande E, Jones D. Examining targets for HIV prevention: intravaginal practices in Urban Lusaka, Zambia. *AIDS Patient Care STDs* 2014;**28**:121–27.

59. Borgdorff H, Tsivtsivadze E, Verhelst R *et al*. Lactobacillus-dominated cervicovaginal microbiota associated with reduced HIV/STI prevalence and genital HIV viral load in African women. *ISME J* 2014;**8**:1781–93.

60. Gautam R, Borgdorff H, Jespers V *et al*. Correlates of the molecular vaginal microbiota composition of African women. *BMC Infect Dis* 2015;**15**:86.

61. Anahtar MN, Byrne EH, Doherty KE *et al*. Cervicovaginal bacteria are a major modulator of host inflammatory responses in the female genital tract. *Immunity* 2015;**42**(**5**):965–76.

62. Robinson CK, Brotman RM, Ravel J. Intricacies of assessing the human microbiome in epidemiologic studies. *Ann Epidemiol* 2016;**26**:311–21.

63. van de Wijgert JH, Borgdorff H, Verhelst R *et al*. The vaginal microbiota: what have we learned after a decade of molecular characterization? *PloS One* 2014;**9**:e105998.

64. van de Wijgert JH, Jespers V. Incorporating microbiota data into epidemiologic models: examples from vaginal microbiota research. *Ann Epidemiol* 2016;**26**:360–65.

65. Seed J, Allen S, Mertens T *et al*. Male circumcision, sexually transmitted disease, and risk of HIV. *J Acquir Immune Defic Syndr* 1995;**8**:83–90.

66. Haaland RE, Hawkins PA, Salazar-Gonzalez J *et al*. Inflammatory genital infections mitigate a severe genetic bottleneck in heterosexual transmission of subtype A and C HIV-1. *PLoS Pathogens* 2009;**5**:e1000274.

67. Price MA, Wallis CL, Lakhi S *et al*. Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in East and Southern Africa. *AIDS Res Hum Retroviruses*. 2011;**27**:5–12.

68. Elema R, Mills C, Yun O, Lokuge K *et al*. Outcomes of a remote, decentralized health center-based HIV/AIDS antiretroviral program in Zambia, 2003 to 2007. *J Int Assoc Physicians AIDS Care (Chic ILL)* 2009;**8**:60–67.

69. Boeras DI, Hraber PT, Hurlston M *et al*. Role of donor genital tract HIV-1 diversity in the transmission bottleneck. *Proc Natl Acad Sci U S A* 2011;**108**:E1156–63.

70. Carlson JM, Schaefer M, Monaco DC *et al*. HIV transmission. Selection bias at the heterosexual HIV-1 transmission bottleneck. *Science* 2014;**345**:1254031.

71. Kempf MC, Allen S, Zulu I *et al*. Enrollment and retention of HIV discordant couples in Lusaka, Zambia. *J Acquir Immune Defic Syndr* 2008;**4**:116–25.