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Circumcision status and incident herpes simplex virus type 2 infection, genital ulcer disease, and HIV infection

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Abstract

Objective—We assessed the protective effect of medical male circumcision (MMC) against HIV, herpes simplex virus type 2 (HSV-2), and genital ulcer disease (GUD) incidence.

Design—Two thousand, seven hundred and eighty-seven men aged 18–24 years living in Kisumu, Kenya were randomly assigned to circumcision (*n*=1391) or delayed circumcision (*n* =1393) and assessed by HIV and HSV-2 testing and medical examinations during follow-ups at 1, 3, 6, 12, 18, and 24 months.

Methods—Cox regression estimated the risk ratio of each outcome (incident HIV, GUD, HSV-2) for circumcision status and multivariable models estimated HIV risk associated with HSV-2, GUD, and circumcision status as time-varying covariates.

Results—HIV incidence was 1.42 per 100 person-years. Circumcision was 62% protective against HIV [risk ratio =0.38; 95% confidence interval (CI) 0.22–0.67] and did not change when controlling for HSV-2 and GUD (risk ratio =0.39; 95% CI 0.23–0.69). GUD incidence was halved among circumcised men (risk ratio =0.52; 95% CI 0.37–0.73). HSV-2 incidence did not differ by circumcision status (risk ratio =0.94; 95% CI 0.70–1.25). In the multivariable model, HIV seroconversions were tripled (risk ratio =3.44; 95% CI 1.52–7.80) among men with incident HSV-2 and seven times greater (risk ratio =6.98; 95% CI 3.50–13.9) for men with GUD.

Conclusion—Contrary to findings from the South African and Ugandan trials, the protective effect of MMC against HIV was independent of GUD and HSV-2, and MMC had no effect on HSV-2 incidence. Determining the causes of GUD is necessary to reduce associated HIV risk and to understand how circumcision confers protection against GUD and HIV

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Conflicts of interest

There are no conflicts of interest.

Keywords

Africa; genital ulcer disease; HIV; herpes simplex virus type 2; Kenya; male circumcision; randomized trial

Introduction

Three randomized controlled trials in Africa demonstrated that adult medical male circumcision (MMC) is effective in reducing HIV acquisition by 50–60% [1–3]. The mechanisms by which this is thought to occur include the following: reduction in HIV target cell populations through removal of mucosal foreskin [4], increased keratinization of penile skin other than the foreskin [5], and reduction of cofactors for infection, such as herpes simplex virus type 2 (HSV-2) and genital ulcer disease (GUD) [6]. In the MMC trial in Rakai, Uganda, circumcision resulted in a 46% reduction in GUD [2] and a 28% reduction in HSV-2 acquisition [7], similar to the 30% reduction in HSV-2 incidence observed among Orange Farm, South Africa, MMC trial participants [8]. An estimated 11% of HIV acquisition among Rakai trial participants may have been due to symptomatic GUD [9], and among Orange Farm trial participants, authors estimated that 28% of HIV infections may have been due to HSV-2 infection [8]. In the Orange Farm trial, the effect of MMC on HIV acquisition was not modified by HSV-2 status [8], whereas in the Rakai trial MMC was not protective against HIV for men who were HSV-2-infected at enrollment [9].

We examined the effect of MMC on HSV-2 and GUD incidence, and whether HSV-2 or GUD modified the protective effect of MMC against HIV acquisition among participants of the randomized trial of MMC to reduce HIV incidence in Kisumu, Kenya.

Methods

The Kisumu trial enrolled 2784 men aged 18–24 years. For inclusion men had to be uncircumcised, HIV-negative, sexually active in the last 12 months, and aged 18–24 years; have a hemoglobin more than 9.0 mmol/l; and reside in Kisumu district. Exclusion riteria included the following: foreskin covering less than half of the glans, a bleeding disorder, keloid formation, other conditions that might unduly increase the risks of elective surgery, or a medical indication for circumcision. Participants with sexually transmitted infections (STIs) or other treatable medical conditions were deferred until treated. Trial recruitment, enrollment, reasons for refusing enrollment, and follow-up have been previously described [1]. Following written informed consent, participants were randomized 1 : 1 to either immediate circumcision or delayed circumcision after a 2-year follow-up period (the control group). Both groups underwent STI and HIV risk reduction counseling and were provided unlimited supplies of free condoms. Personal interview, medical examination, and laboratory testing for HIV were conducted at baseline, 1, 3, 6, 12, 18, and 24 months from randomization for both the circumcision and the control groups. The study was approved by the Institutional Review Boards of the University of Illinois at Chicago, the Kenyatta National Hospital, RTI International, and University of Manitoba and was overseen by a Data and Safety Monitoring Board (DSMB).

Detection of herpes simplex virus type 2 and syphilis

Specimens were collected for HSV-2 and syphilis testing at baseline, 6, 12, 18, and 24 months from randomization for all participants. Serum specimens were tested for HSV-2 antibody (Kalon HSV-2 IgG ELISA; Kalon Biological Limited, Aldershot, UK), using the manufacturer’s recommended cut-off. In men who were initially HSV-2-seronegative who seroconverted to HSV-2, the last available sample was tested, and then previous samples were tested to determine the visit at which HSV-2 seroconversion occurred. Syphilis infection was assessed at each study visit using the rapid plasma reagin test (RPR) (Macro-Vue; Becton Dickinson, New Jersey, USA), confirmed by the *Treponema pallidum* hemagglutination (TPHA) assay (Randox Laboratories Ltd., Ardmore, UK). All genital ulcers were tested for *Haemophilus ducreyi* by culture. Testing was conducted at the study clinic and the University of Nairobi Department of Medical Microbiology research laboratory. A random sample of clinically identified genital ulcers were tested for *H. ducreyi*, *T. pallidum* and HSV (did not distinguish between type 1 and type 2) by multiplex PCR at the University of Manitoba Department of Medical Microbiology research laboratory [10].

Self-reported and physical examination findings of genital ulcer disease

All consenting participants underwent standardized medical examination and history at all planned study visits [1]. Participants were asked about the presence of painless sores and painful sores occurring in the genital region in the past 6 months and at the current visit. At all planned study visits, all participants underwent genital examination by trained clinicians, who recorded the presence or absence of genital ulcers, and the location and number of ulcers. Due to the broad range of clinical presentation for ulcerative infections in the genital region, a restricted definition was not used and clinicians were instructed to record any epithelial defect in the skin or mucosa of the genitalia.

HIV testing

Testing for HIV infection was conducted using a parallel double rapid test protocol, using Determine HIV 1/2 (Abbott Diagnostic Division, Hoofddorp, The Netherlands), and the Uni-Gold Recombigen HIV Test (Trinity Biotech, Wicklow, Ireland). Men who were concordant negative were eligible for the study. Concordant positive results were confirmed by double ELISA and men were informed of their HIV status and followed-up at the study clinic or at the New Nyanza Provincial Hospital. Men with discordant results were followed up with additional tests to determine their HIV status, but were not enrolled. For determination of HIV seroconversion for analysis, positive rapid test and ELISA test results were confirmed by Health Canada National HIV Reference Laboratory (Ottawa, Ontario, Canada) by line immunoassay (INNO-LIA HIV 1/2; Immunogenetics NV, Ghent, Belgium). Specimens indeterminate by line immunoassay were tested by PCR at Health Canada or the Fred Hutchinson Cancer Research Center (Seattle, Washington, USA), with the PCR result deemed to be definitive. At the time of the DSMB meeting that halted the trial and in our published primary results [1], we reported that four men had been found to be HIV-positive at baseline after detailed testing. After the article was published, it was determined that two of the men who were labeled HIV-positive at baseline in the circumcision group were HIV-negative (therefore, needing to be included in the analysis as HIV-negative). Also, it was determined that one of the men who had been labeled HIV-positive in the circumcision group at 3 months was positive at baseline (therefore, needing to be excluded from analyses of HIV seroconversion). Also, one individual in the control group labeled positive at 12 months was determined negative (therefore, remaining in analysis, but with a different outcome). Our current analysis reflects these updated results, which have been previously presented [11].

Statistical analysis

Incident HSV-2 was defined as detection of HSV-2 antibody subsequent to a negative HSV-2 antibody test result at enrollment. Incident syphilis was defined as having a positive RPR test with positive TPHA test, subsequent to a negative RPR and negative TPHA test at enrollment. Men with baseline syphilis infection (n =27) were excluded to eliminate any potential uncertainty that may have arisen from whether these were truly new infections or whether these were baseline infections that were treatment failures. Prior exposure to syphilis was defined as being RPR-negative with a positive TPHA. GUD was defined as having physical examination findings, or a current or past 6 months complaint of genital sores. As exposures, incident syphilis, GUD, and HSV-2 were treated as time-varying states in Cox regression models. As outcomes, observation was censored at first incidence of syphilis or GUD and at HSV-2 seroconversion. HIV seroconversion was defined as concordant positive results from the parallel test protocol. Circumcision status was analyzed as a fixed covariate based on treatment group (intention to treat, ITT) and as a time-varying covariate by status (as treated). Because this was a secondary analysis and the trial was not designed to examine HSV-2 and GUD as endpoints, we present the results of as treated analysis. The interpretation of results of ITT and as treated analyses did not differ and only as treated results are presented (results of ITT analysis available from the authors). Thus, results refer to circumcision by status rather than assignment or individual.

There were 2784 men enrolled in the trial, with 1391 randomized to circumcision and 1393 randomized to control. The trial's target sample size was 2776 (1388 in each group) to detect a 50% difference in 2-year HIV seroincidence between the treatment groups [1]. This analysis excluded three men found to be HIV-infected at baseline after detailed testing, three men who were found to be outside the inclusion age range, and 32 men with no follow-up. A total of 16 of the 1378 men in the control group who were included in this analysis were circumcised during the 24 months of the trial.

Results

Baseline characteristics

As previously reported, the control and treatment arms were well balanced regarding sociodemographic details, behavioral characteristics, baseline STI prevalences, and rates of follow-up [1]. Baseline prevalences of HSV-2, active syphilis, and GUD were similar between study arms (Table 1). Of 2748 men with HSV-2 testing at enrollment, 727 (26.5%) men were seropositive, 59 (8.1%) of whom had GUD (by examination or history). Among 2021 HSV-2-seronegative men at baseline, 49 (2.4%) had GUD. Among 2741 men tested for syphilis at enrollment, 27 (1.0%) were positive, of whom three (11.1%) had GUD. Overall, 108 (3.9%) men had GUD at enrollment: 57 (52.8%) who were HSV-2-positive only, one (0.9%) with syphilis only, two (1.9%) with HSV-2 and syphilis, and 48 (44.4%) with neither HSV-2 nor syphilis. No men had ulcers that were positive for *H. ducreyi* at baseline or at follow-up.

Medical male circumcision and herpes simplex virus type 2 incidence

Over follow-up, there was no difference in HSV-2 incidence by circumcision status. Among men who were HSV-2-seronegative at baseline, the cumulative incidence of HSV-2 was 5.8 per 100 person-years among circumcised men and 6.1 per 100 person-years among uncircumcised men [risk ratio =0.94; 95% confidence interval (CI) 0.70–1.25] (Table 2). The results reported here are based on the manufacturer's suggested cutoff value, specifically greater than 1.1 on optical density index for a positive test. However, no differences were found between circumcised and uncircumcised at other cutoff values of 1.5, 2.0, 2.5, 3.0, or 3.5.

Medical male circumcision and genital ulcer disease

Over follow-up, the incidence of GUD was 3.94 cases per 100 person-years and was nearly halved (risk ratio =0.52; 95% CI 0.37–0.73) among circumcised compared with uncircumcised men (2.7 vs. 5.2 per 100 person-years). The protective effect of MMC against GUD incidence over follow-up did not differ by whether men were HSV-2-seropositive at enrollment (risk ratio =0.57; 95% CI 0.35–0.95) or remained HSV-2-seronegative throughout follow-up (risk ratio =0.55; 95% CI 0.31–0.97), but was more pronounced among men who acquired HSV-2 over follow-up (risk ratio =0.33; 95% CI 0.15–0.75). Recurrent GUD did not differ by circumcision status among men who were HSV-2-positive at baseline: 11 of 362 (3%) circumcised men who were HSV-2-positive at baseline had two or more GUD incidences compared with 14 of 365 (4%) uncircumcised men who were HSV-2-positive at baseline.

Swabs for multiplex PCR were obtained from visible lesions in 14 circumcised men and 80 uncircumcised men. HSV was detected in 36% of lesions from circumcised men and 30% of lesions from uncircumcised men. *T. pallidum* was detected in 7% of lesions from circumcised men and 4% of lesions from uncircumcised men, and no *H. ducreyi* was detected. Thus, by PCR 66% of genital ulcers did not have detectable STI-associated pathogens [57% (n =8) circumcised men vs. 68% (n =54) uncircumcised men]. Of those men with no pathogen recovered by PCR (n =62), 26 (42%) were HSV-2-seropositive and none were RPR-positive. Therefore, overall, 38% (36 of 94) of GUD specimens had no STI-associated pathogen by serology or PCR.

At baseline, there was no difference in the distribution of genital ulcer locations by study arm (Table 3A). On follow-up, genital ulcers were more likely to be multiply located in uncircumcised than circumcised men [39.3% (33 of 84) vs. 14.3% (five of 35), P =0.008), and 49% (61 of 124) of ulcers in uncircumcised men occurred on the prepuce [73% (61 of 84) of uncircumcised men with ulcers had preputial ulcers] (Table 3B). Of nonpreputial ulcers, ulcers more commonly occurred on the glans and corona of uncircumcised men, whereas ulcers were commonly found on the proximal shaft of circumcised men (Table 3B).

Medical male circumcision and syphilis incidence

Among 2714 men who did not have syphilis at enrollment, 13 developed syphilis during follow-up (six among uncircumcised men and seven among circumcised men), and this did not differ by circumcision status (0.4 per 100 person-years circumcised vs. 0.3 per 100 person-years uncircumcised men, risk ratio =1.23; 95% CI 0.41–3.65). Syphilis incidence was not included in the multivariate models because there were too few cases for interpretation.

Medical male circumcision and HIV incidence, adjusting for herpes simplex virus type 2 serostatus and genital ulcer disease

Controlling for baseline HSV-2 serostatus, HSV-2 acquisition over follow-up, GUD at baseline, and GUD over follow-up, the protective effect of MMC against HIV acquisition was 61% (risk ratio =0.39; 95% CI 0.23–0.69) (Table 4A). In the multivariable model, baseline HSV-2 infection and baseline GUD were not statistically significantly associated with HIV risk, whereas incident HSV-2 infection more than tripled the risk of HIV acquisition (risk ratio =3.44; 95% CI: 1.52–7.80). Controlling for HSV-2 serostatus and circumcision status, GUD during follow-up increased the risk of HIV acquisition by seven-fold (risk ratio =6.98; 95% CI 3.50–13.9).

Results were similar when restricted to men who were HSV-2-seronegative at baseline (Table 4B): the protective effect of MMC against HIV was 68% (risk ratio =0.32; 95% CI 0.15–0.68). Adjusted for GUD at baseline, and for GUD during follow-up and HSV-2 incidence, the protective effect of MMC against HIV was unchanged (risk ratio =0.35; 95% CI 0.17–0.75). Controlling for MMC, HIV risk nearly tripled among men with incident HSV-2 (risk ratio =2.78; 95% CI 1.18–6.54) and was over 14-fold higher among men with GUD during follow-up (risk ratio =14.8; 95% CI 6.54–33.7).

Sequence of herpes simplex virus type 2, genital ulcer disease, and HIV infections

In 20 (32%) of 63 HIV seroconversions [16 of 46 (35%) uncircumcised, four of 17 (24%) circumcised], HIV was not preceded or followed by HSV-2 or GUD. HIV was preceded by HSV-2 and/or GUD in 34 of 63 (54%) seroconversions; seven of 63 (11%) HIV seroconversions were detected in the same interval as HSV-2 and/or GUD; and HIV seroconversion preceded HSV-2/GUD in two of 63 (3%) cases (Table 5). Where HIV seroconversion followed HSV-2 and/or GUD, the median time from HSV-2/GUD detection to HIV seroconversion was 12 months for both circumcised men and uncircumcised men.

Discussion

In our study, the protective effect of MMC against HIV acquisition did not change when adjusting for baseline and incident HSV-2 or GUD, indicating that the protective effect of MMC on HIV acquisition could not be explained by the effects of MMC on HSV-2 or GUD. This is in contrast to findings from the Rakai trial in which 11.2 and 8.6% of the protective effect of circumcision against HIV was mediated by reductions in GUD and HSV-2 [9], respectively, and the Orange Farm trial in which 28% of the protective effect of MMC against HIV was attributed to reductions in HSV-2 [8].

Unlike the trials in Orange Farm and Rakai, our study did not find that MMC provided protection against HSV-2 acquisition, which could be due to the location of lesions or test performance. A meta-analysis of the association between male circumcision and HSV-2 from observational studies by Weiss *et al.* [12] found a modest protective effect (12%) that was marginally statistically significant. Unlike HIV, transmission of HSV-2 can result from contact between skin and mucosa throughout the genital region [13] or may be acquired orally [14], and this may render HSV-2 acquisition less dependent upon the presence of foreskin mucosa. Among circumcised men, 37% of clinically detected genital ulcers were detected on the penile shaft. Comparable results on lesion location are not reported for the MMC trials in Rakai and Orange Farm. Although the sensitivity (95%) and specificity (91%) of the Kalon test for detecting HSV-2 in sub-Saharan Africa are high [15], we found a lower sensitivity (92%) and specificity (79%) in a validation study among men aged 18–24 years in Kisumu [16]. This amount of misclassification could obscure a small effect of MMC on HSV-2 acquisition.

The relative reduction in GUD among circumcised men was similar for those who remained HSV-2-seronegative throughout follow-up compared with those who were HSV-2-seropositive. In a sample of genital ulcers, 38% were not associated with HSV or *T. pallidum* by PCR or serology. In the Rakai trial, 58% of clinically detected GUD did not have an STI-associated pathogen [9] and an ulcerative STI pathogen was not detected in 32% of GUD specimens in female sex partners [17]. Similar to our finding in Kenya, in a prospective study of HIV-negative men in India by Reynolds *et al.* [18], genital ulcer on physical examination at follow-up was more than halved among circumcised men, yet the risk of HSV-2 acquisition did not differ by circumcision status. In a prospective evaluation of HIV incidence among male STI clinic patients in Kenya who presented with either urethritis or GUD, circumcision and GUD were risks for HIV seroconversion and the effect sizes were not modified when adjusted for each other [19]. The authors note that it is unlikely that the increased risk of HIV seroconversion associated with being uncircumcised is explained by GUD as an intermediary variable. Observational studies have found the proportion of genital ulcers without STI causes in men and women to be 27–39% in Kenya [20], South Africa [21], Botswana [22], Madagascar [23], and Tanzania [24]. Outside of sub-Saharan Africa, approximately one-third of STI clinic patients presenting with GUD in studies from France [25] and India [26] had no ulcerative STI pathogen.

If MMC provided protection against herpetic GUD, we would expect to see a reduction in genital ulcers for HSV-2-seropositive circumcised men compared with uncircumcised men, with no reduction in GUD among HSV-2-seronegative men who were circumcised. If MMC provided protection against both herpetic and nonherpetic ulcers, then we would expect to observe a stronger protective effect of MMC against GUD in HSV-2-seropositive men, resulting from the protection against herpetic GUD in addition to protection against non-herpetic GUD. A significant proportion of genital ulcerations may be associated with HSV-1 [13]. However, it is difficult to hypothesize how MMC may reduce HSV-1-associated GUD but not HSV-2-associated GUD. Although the lack of identification of HSV-2 and other STIs in genital ulcers may be attributed to false-negative results of assays with imperfect sensitivity, poor specimen collection, and stage of disease, alternative explanations have rarely been considered. Other potential causes of an ulcer-like appearance may include mechanical trauma to the epithelium, with or without secondary infection. Minor penile trauma, such as abrasions and cuts, has been cited as a possible mechanism for increased HIV acquisition in uncircumcised men [6,27,28]. In our cohort, self-reported penile coital injuries (defined as scratches, cuts, abrasions, soreness, or bleeding of the penis during sex) were present in 65% of men at baseline and were reported by 30–40% of men at 24 months of follow-up [29]. The odds of self-reported penile coital injuries were reduced by 39% ($P < 0.001$) among men who underwent circumcision compared with those remaining uncircumcised. In addition to physical trauma, epithelial and mucosal barrier disruptions may result in bacterial infections and dermatoses. In a case–control study of 662 men seeking diagnosis and management of genital skin disease, uncircumcised men had 3.2 times increased odds of genital dermatoses compared with circumcised men in adjusted analyses [30]. Specific dermal conditions found more often among uncircumcised men were psoriasis, lichen sclerosis, and seborrheic dermatitis. In addition to exacerbating epithelial disruptions, bacterial infections may induce genital ulcerations. To our knowledge, no studies have broadly investigated ‘non-STI’ causes of genital ulcers. We conducted multitag pyrosequencing of the 16S rRNA gene on 59 of the 94 genital ulcer specimens and found specific and statistically significant associations between the genital ulcers with non-STI causes and the presence of anaerobic bacteria that are associated with periodontal disease [31].

As trauma and potentially other causes of ulcer-like lesions (e.g., ulcerative or erosive balanitis) are more common among uncircumcised men and may be classified as ‘genital ulcers’, a reduction of these other causes may explain the reduction in non-HSV-2 GUD associated with circumcision. Regardless of the causes of these ulcers, the consistent protective effect of MMC against GUD found in our trial in Kisumu (48%) and in the Rakai trial (46%) indicate that MMC can be recommended to reduce GUD incidence. Although 35% of HIV seroconversions occurred in the absence of or prior to HSV-2 infection or GUD, 54% of HIV seroconversions were preceded by HSV-2 infection and/or GUD. The increased risk of HIV acquisition and transmission associated with HSV-2 and GUD is well known, but the extremely high risk associated with GUD, adjusted for circumcision status and HSV-2, are among the highest observed. Simulations to model the early spread of HIV in sub-Saharan Africa estimate that GUD was paramount for HIV emergence and lack of circumcision was secondary [32]. Determining the causes of genital ulcers will be crucial to effective HIV prevention: among HIV-positive men enrolled in a randomized trial of episodic acyclovir to reduce lesional HIV viral load, in multivariable analysis, the odds of HIV viral shedding was 40% lower from HSV-2 positive ulcers compared with ulcers with unknown etiology [33].

Conclusion

In the Kenyan randomized controlled trial of MMC for HIV prevention, the protective effect of MMC on HIV acquisition was independent of effects of MMC on HSV-2 and GUD, indicating that the mechanism of protection was not through reduction of these cofactors. Given the three-fold higher risk of HIV acquisition among men with incident HSV-2, strategies are needed to reduce HSV-2 in men. Reducing HSV-2 acquisition in women, through approaches such as tenofovir gel if Center for the AIDS Programme of Research in South Africa 004 Trial results are confirmed [34], may be one approach. MMC led to a 48% reduction in GUD that is largely nonherpetic. Determining the causes of these ulcers will be crucial to reducing and preventing GUD, and the associated risk of HIV acquisition and transmission. Independent of GUD and HSV-2, the protective efficacy of MMC against HIV acquisition was approximately 60%, suggesting that varying incidences of HSV-2 and GUD in different populations will likely not affect the overall efficacy of circumcision.

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Study concept and design: R.C.B., S.M.

Acquisition of data: K.A., R.C.B., I.M., S.M., C.B.P.

Drafting of the manuscript: S.D.M.

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Table 1

Selected baseline characteristics by treatment assignment.

Characteristic	**Intervention arm (*N* =1371), *n/N* (%)**	**Control arm (*N* =1378), *n/N* (%)**
HSV-2 seropositive	384/1370 (28.0)	343/1378 (24.9)
No GUD	356 (92.7)	312 (91.0)
GUD	28 (7.3)	31 (9.0)
HSV-2-seronegative	986/1370 (72.0)	1035/1378 (75.1)
No GUD	955 (96.9)	1017 (98.3)
GUD	31 (3.1)	18 (1.7)
Active syphilis	18/1368 (1.3)	9/1373 (0.7)
No GUD	16 (88.9)	8 (88.9)
GUD	2 (11.1)	1 (11.1)
Genital ulcer disease	59/1371 (4.3)	49/1378 (3.6)
HSV-2 seropositive and syphilis	1 (1.7)	1 (2.0)
HSV-2 seropositive only	27 (45.8)	30 (61.2)
Syphilis only	1 (1.7)	0 (0.0)
No causes	30 (50.8)	18 (36.7)

GUD, genital ulcer disease (detected by physical examination or by patient report); HSV-2, herpes simplex virus type 2.

Table 2

Incident herpes simplex virus type 2, syphilis, and genital ulcer disease by circumcision status over 24 months.

Characteristic	Cases/person-years	Incidence per 100 person-years	Risk ratio (95% CI) circumcised to uncircumcised
HIV incidence			
Circumcised	17/2166.7	0.8	0.38 (0.22, 0.67)
Uncircumcised	46/2258.3	2.0	*P* <0.001
Incident HSV-2[a]			
Circumcised	86/1493.5	5.8	0.94 (0.70, 1.25)
Uncircumcised	100/1628.5	6.1	*P* =0.655
Incident syphilis[b]			
Circumcised	7/1897.5	0.4	1.23 (0.41, 3.65)
Uncircumcised	6/1976.0	0.3	*P* =0.714
GUD after enrollment			
Circumcised	51/1912.0	2.7	0.52 (0.37, 0.73)
Uncircumcised	101/1950.0	5.2	*P* <0.001
GUD after enrollment among HSV-2-positive individuals at enrollment			
Circumcised	26/524.5	5.0	0.57 (0.35, 0.95)
Uncircumcised	40/472.0	8.5	*P* =0.029
GUD after enrollment among HSV-2-negative individuals throughout follow-up			
Circumcised	18/1262.0	1.4	0.55 (0.31, 0.97)
Uncircumcised	35/1349.5	2.6	*P* =0.039
GUD after enrollment among HSV-2-positive individuals during follow-up			
Circumcised	8/119.0	6.7	0.33 (0.15, 0.75)
Uncircumcised	25/125.5	19.9	*P* =0.008

Cases are attributed to circumcised or uncircumcised status at the time detected positive. Person-years in circumcised and uncircumcised states are reported in which a participant may have some of the follow-up time in both states. The risk ratio is estimated from a Cox model with time-dependent circumcision status at 6, 12, 18, and 24 months. CI, confidence interval; GUD, genital ulcer disease; HSV-2, herpes simplex virus type 2.

[a]HSV-2 incidence is computed among participants who were HSV-2-seronegative at enrollment.

[b]Syphilis incidence is computed among participants without syphilis detected prior to enrollment.

Table 3

Location of genital ulcers on exams among treatment group subsets[a].

Location[b]	Circumcised, *n* (%)	Uncircumcised, *n* (%)	*P*-value
(A) Location of genital ulcers prior to randomization			
Prepuce	21 (1.6)	13 (0.9)	0.123
Glans	6 (0.4)	5 (0.3)	0.768
Corona	11 (0.8)	4 (0.2)	0.069
Proximal shaft	2 (0.1)	0 (0.0)	0.234
Distal shaft	1 (0.0)	0 (0.0)	0.484
Scrotum	0 (0.0)	1 (0.0)	1.000
Inguinal Region	0 (0.0)	0 (0.0)	1.000
Number with examination	1304	1393	
(B) Location of genital ulcers on follow-up examinations			
Prepuce	0 (0.0)	61 (4.5)	<0.001
Glans	5 (0.3)	22 (1.6)	0.002
Corona	19 (1.5)	34 (2.5)	0.071
Proximal shaft	9 (0.7)	3 (0.2)	0.084
Distal shaft	6 (0.4)	2 (0.1)	0.168
Scrotum	1 (0.0)	1 (0.0)	1.000
Inguinal region	1 (0.0)	1 (0.0)	1.000
Number with examination	1255	1331	

[a]The circumcised group subset is restricted to men in the circumcision group who were circumcised within 2 weeks of randomization. The uncircumcised group subset is all men in the control group for the duration of time uncircumcised.

[b]Locations are not mutually exclusive; ulcers may occur in multiple locations.

Table 4

A: HIV risk ratios [95% confidence interval] by HSV-2, genital ulcer disease and circumcision status.

Covariate	Unadjusted	Adjusted for circumcision status	Adjusted for all covariates shown
Overall (*N* =2749)			
HSV-2 at baseline	1.74 [1.05–2.90] *P* =0.033	1.82 [1.09–3.03] *P* =0.022	0.54 [0.24–1.24] *P* =0.146
Time-dependent HSV-2 status	2.37 [1.44–3.89] *P* <0.001	2.47 [1.50–4.05] *P* <0.001	3.44 [1.52–7.80] *P* =0.003
Time-dependent GUD	9.87 [5.05–19.3] *P* <0.001	8.97 [4.58–17.6] *P* <0.001	6.98 [3.50–13.9] *P* <0.001
Time-dependent circumcision status	0.38 [0.22–0.67] *P* <0.001	NA	0.39 [0.23–0.69] *P* =0.001
Among men who were HSV-2 negative at baseline (*N* =2021)			
Incident HSV-2	4.31 [1.93–9.62] *P* <0.001	4.37 [1.95–9.77] *P* <0.001	2.78 [1.18–6.54] *P* =0.020
Time-varying incident GUD	21.5 [9.84–46.8] *P* <0.001	18.9 [8.62–41.3] *P* <0.001	14.8 [6.54–33.7] *P* <0.001
Time-dependent circumcision status	0.32 [0.15–0.68] *P* =0.003	NA	0.35 [0.17–0.75] *P* =0.007

The risk ratio for HIV is estimated from a Cox model with fixed and time-dependent covariates as described. CI, confidence interval; GUD, genital ulcer disease; HSV-2, herpes simplex virus type 2.

Table 5

Sequence of herpes simplex virus type 2, genital ulcer disease, and HIV seroconversion among men with HIV seroconversion.

	Uncircumcised (*N* =30), *n* (%)	**Circumcised (*N* =13), *n* (%)**
HSV-2 and/or GUD before HIV	25 (83)	9 (69)
HSV-2 only then HIV	18	7
GUD only then HIV	6	0
HSV-2 and GUD before HIV	1	2
HIV then GUD and/or HSV-2	2 (7)	0 (0)
HIV at same time as HSV-2 and/or GUD	3 (10)	4 (31)

GUD, genital ulcer disease; HSV-2, herpes simplex virus type 2.