Circumcision Status and Incident HSV-2 Infection, Genital Ulcer Disease, and HIV Infection

[1]Supriya D. Mehta, PhD

[2]Stephen Moses, MD

[3]Corette B. Parker, DrPH

[4]Kawango Agot, PhD

[5]Ian Maclean, PhD

[1]Robert C. Bailey, PhD

[1] Department of Epidemiology and Biostatistics, University of Illinois Chicago School of Public Health, Illinois, USA

[2] Departments of Medical Microbiology, Community Health Sciences and Medicine, University of Manitoba, Winnipeg, Canada

[3] RTI International, Research Triangle Park, North Carolina, USA

[4] UNIM Project, Kisumu, Kenya

[5] Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada

Corresponding Author: Dr. Supriya Mehta, 958 SPHPI M/C 923; 1603 W. Taylor St.; Chicago, IL 60622; phone: (312) 413-7485; fax (312) 996-0064; email: supriyad@uic.edu

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Principal Contributions of Authors

Study concept and design: Bailey, Moses, Ndinya-Achola

Acquisition of data: Agot, Bailey, Maclean, Moses, Ndinya-Achola, Parker

Drafting of the manuscript: Mehta

Critical revision of the manuscript for important intellectual content: Agot, Bailey, Maclean, Mehta, Moses, Parker

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Abstract (Word Count = 243)

Objective: We assessed the protective effect of medical male circumcision (MMC) against HIV, HSV-2, and GUD incidence.

Design: 2784 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 (RR) 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 [RR=0.38; 95% CI: 0.22 - 0.67], and did not change when controlling for HSV-2 and GUD [RR=0.39; 95% CI: 0.23 - 0.69]. GUD incidence was halved among circumcised men [RR=0.52, 95% CI: 0.37 - 0.73]. HSV-2 incidence did not differ by circumcision status [RR=0.94; 95% CI: 0.70 - 1.25]. In the multivariable model, HIV seroconversions were tripled [RR=3.44; 95% CI: 1.52 - 7.80] among men with incident HSV-2 and 7 times greater [RR=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.

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: 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 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 *Herpes simplex* virus type 2 (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 2,784 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 > 9·0 mmol/L; and reside in Kisumu District. Exclusion criteria included: 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 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 two-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 HSV-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, United Kingdom), 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, United States), confirmed by the *Treponema pallidum* hemagglutination

(TPHA) assay (Randox Laboratories Ltd., Ardmore, United Kingdom). 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 polymerase chain reaction (PCR) at the University of Manitoba Department of Medical Microbiology research laboratory[10].

Self-Reported and Physical Examination Findings of GUD

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 six 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, 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, WA, 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 4 men had been found to be HIV positive at baseline after detailed testing. After the paper was published, it was determined that 2 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 subject 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. Genital ulcer disease 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, AT). 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 AT analysis. The interpretation of results of ITT and AT analyses did not differ and only AT 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 2,784 men enrolled in the trial, with 1,391 randomized to circumcision and 1,393 randomized to control. The trial's target sample size was 2,776 (1,388 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 1,378 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 socio-demographic 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 2,748 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 2,021 HSV-2 seronegative men at baseline, 49 (2·4%) had GUD. Among 2,741 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.

MMC and HSV-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 (P-Ys) among circumcised men and 6.1 per 100 P-Ys among uncircumcised men [RR=0·94; 95% CI: 0·70-1·25] (Table 2). The results reported here are based on the manufacturer's suggested cutoff value, specifically >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.

MMC and GUD

Over follow-up, the incidence of GUD was 3·94 cases per 100 P-Ys, and was nearly halved [RR=0·52; 95% CI: 0·37 – 0.73] among circumcised compared to uncircumcised men [2·7 per

100 P-Ys vs. 5·2 per 100 P-Ys]. The protective effect of MMC against GUD incidence over follow-up did not differ by whether men were HSV-2 seropositive at enrollment [RR=0·57; 95% CI: 0·35 - 0·95] or remained HSV-2 seronegative throughout follow-up [RR=0·55; 95% CI: 0·31 - 0·.97], but was more pronounced among men who acquired HSV-2 over follow-up [RR=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: 11/362 (3%) circumcised men who were HSV-2 positive at baseline had two or more GUD incidences compared to 14/365 (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% (36/94) of GUD specimens had no STI associated etiology 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% [33/84] vs. 14·3% [5/35], p = 0.008), and 49% (61/124) of ulcers in uncircumcised men occurred on the prepuce (73% [61/84] of uncircumcised men with ulcers had preputial ulcers) (Table 3B). Of non-preputial ulcers, ulcers more

commonly occurred on the glans and corona of uncircumcised men, while ulcers were commonly found on the proximal shaft of circumcised men (Table 3B).

MMC and Syphilis Incidence

Among 2,714 men who did not have syphilis at enrollment, 13 developed syphilis during follow-up (6 among uncircumcised men and 7 among circumcised men), and this did not differ by circumcision status [0·4 per 100 P-Ys circumcised vs. 0·3 per 100 P-Ys uncircumcised men, RR=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.

MMC and HIV Incidence, Adjusting for HSV-2 Serostatus and GUD

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% [RR=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, while incident HSV-2 infection more than tripled the risk of HIV acquisition [RR=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 7-fold [RR=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% [RR=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 [RR=0·35; 95% CI: 0·17 - 0·75].

Controlling for MMC, HIV risk nearly tripled among men with incident HSV-2 [RR=2·78; 95% CI: 1·18 – 6·54], and was over 14-fold higher among men with GUD during follow-up [RR=14·8; 95% CI: 6·54 – 33·7].

Sequence of HSV-2, GUD, and HIV Infections

In 20 (32%) of 63 HIV seroconversions (16/46 [35%] uncircumcised, 4/17[24%] circumcised), HIV was not preceded or followed by HSV-2 or GUD. HIV was preceded by HSV-2 and/or GUD in 34/63 (54%) seroconversions; 7/63 (11%) HIV seroconversions were detected in the same interval as HSV-2 and/or GUD; and HIV seroconversion preceded HSV-2/GUD in 2/63 (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, where 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, where 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. found a modest protective effect (12%) that was marginally statistically significant.[12] Unlike HIV, transmission of HSV-2 can result from contact between skin or 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 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 to 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., genital ulcer on physical exam at follow-up was more than halved among circumcised men, yet the risk of HSV-2 acquisition did not differ by circumcision status.[18] In a prospective evaluation of HIV incidence among male STD 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 etiology 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 to uncircumcised men, with no reduction in GUD among HSV-2 seronegative men who were circumcised. If MMC provided protection against both herpetic and non-herpetic 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. While 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 follow-up.[29] The odds of self-reported penile coital injuries were reduced by 39% ($p<0.001$) among men who underwent circumcision compared to 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 to 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 etiology 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 etiology 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. While 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 etiology 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 to ulcers with unknown etiology.[33]

Conclusions

In the Kenyan randomized controlled trial of MMC for HIV prevention, the protective effect of medical male circumcision 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 co-factors. Given the 3-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 CAPRISA 004 results are confirmed[34], may be one approach. MMC led to a 48% reduction in GUD that is largely non-herpetic. Determining the etiology 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.

References

1. Bailey RC, Moses S, Parker CB, et al. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. *Lancet* 2007;369:643-56.
2. Gray RH, Kigozi G, Serwadda D, et al. Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial. *Lancet* 2007;369:657-66.
3. Auvert B, Taljaard D, Lagarde E, Sobngwi-Tambekou J, Sitta R, Puren A. Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. *PLoS Med* 2005;2(11):e298. Epub 2005 Oct 25.
4. Hirbod T, Bailey RC, Agot K, et al. Abundant expression of HIV target cells and C-type lectin receptors in the foreskin tissue of young Kenyan men. *Am J Pathol* 2010;176:2798-805.
5. McCoombe SG, Short RV. Potential HIV-1 target cells in the human penis. *AIDS* 2006;20:1491-5.
6. Szabo R, Short RV. How does male circumcision protect against HIV infection? *BMJ* 2000; 320:1592–1594.
7. Tobian AAR, Serwadda D, Quinn TC, et al. Male circumcision for the prevention of HSV-2 and HPV infections and syphilis. *N Engl J Med* 2009;360:1298-1309.
8. Sobngwi-Tambekou J, Taljaard D, Lissouba P, Zarca K, Puren A, Lagarde E, Auvert B. Effect of HSV-2 serostatus on acquisition of HIV by young men: results of a longitudinal study in Orange Farm, South Africa. *J Infect Dis* 2009;199:958-64.
9. Gray RH, Serwadda D, Tobian AAR, et al. Effects of genital ulcer disease and herpes simplex virus type 2 on the efficacy of male circumcision for HIV prevention: Analyses from the Rakai trials. *PLoS Med* 2009;6(11):e1000187.

10. Orle KA, Gates CA, Martin DH, Body BA, Weiss JB. Simultaneous PCR detection of *Haemophilus ducreyi*, *Treponema pallidum*, and herpes simplex virus types 1 and 2 from genital ulcers. *J Clin Microbiol* 1996;34:49-54.
11. Bailey RC, Moses S, Ndinya-Achola JO, et al. The protective effect of male circumcision is sustained for at least 42 months: Results from the Kisumu, Kenya trial. 17th Annual International AIDS Conference, August 3-8, 2008, Mexico City.
12. Weiss HA, Thomas SL, Munabi SK, Hayes RJ. Male circumcision and risk of syphilis, chancroid, and genital herpes: a systematic review and meta-analysis. *Sex Transm Infect* 2006;82:101-110.
13. Gupta R, Warren T, Wald A. Genital herpes. *Lancet* 2007;370:2127-37.
14. Kim HN, Meier A, Huang ML, et al. Oral herpes simplex virus type 2 reactivation in HIV-positive and -negative men. *J Infect Dis* 2006;194:420-7.
15. Biraro S, Mayaud P, Morrow RA, Grosskurth H, Weiss HA. Performance of commercial herpes simplex virus type-2 antibody tests using serum samples from Sub-Saharan Africa: a systematic review and meta-analysis. *Sex Transm Dis* 2011;38:140-7.
16. Smith JS, Bailey RC, Westreich DJ, et al. Herpes simplex virus type 2 antibody detection performance in Kisumu, Kenya, using the Herpeselect ELISA, Kalon ELISA, Western blot and inhibition testing. *Sex Transm Infect* 2009;85:92-6.
17. Brankin AE, Tobian AA, Laeyendecker O, et al. Aetiology of genital ulcer disease in female partners of male participants in a circumcision trial in Uganda. *Int J STD AIDS* 2009;20:650-1.
18. Reynolds SJ, Shepherd ME, Risbud AR, et al. Male circumcision and risk of HIV-1 and other sexually transmitted infections in India. Lancet. 2004 Mar 27;363(9414):1039-40.

19. Nasio JM, Nagelkerke NJ, Mwatha A, Moses S, Ndinya-Achola JO, Plummer FA. Genital ulcer disease among STD clinic attenders in Nairobi: association with HIV-1 and circumcision status. *Int J STD AIDS* 1996;7:410-4.
20. Plummer FA, D'Costa LJ, Nsanze H, Karasira P, MacLean IW, Piot P, Ronald AR. Clinical and microbiologic studies of genital ulcers in Kenyan women. *Sex Transm Dis* 1985;12:193-7.
21. Moodley P, Sturm PD, Vanmali T, Wilkinson D, Connolly C, Sturm AW. Association between HIV-1 infection, the etiology of genital ulcer disease, and response to syndromic management. *Sex Transm Dis* 2003;30:241-5.
22. Paz-Bailey G, Rahman M, Chen C, et al. Changes in the etiology of sexually transmitted diseases in Botswana between 1993 and 2002: implications for the clinical management of genital ulcer disease. *Clin Infect Dis* 2005;41:1304-12.
23. Behets FM, Andriamiadana J, Randrianasolo D, et al. Chancroid, primary syphilis, genital herpes, and lymphogranuloma venereum in Antananarivo, Madagascar. *J Infect Dis* 1999;180:1382-5.
24. Ahmed HJ, Mbwana J, Gunnarsson E, et al. Etiology of genital ulcer disease and association with human immunodeficiency virus infection in two Tanzanian cities. *Sex Transm Dis* 2003;30:114-9.
25. Hope-Rapp E, Anyfantakis V, Fouéré S, et al. Etiology of genital ulcer disease. A prospective study of 278 cases seen in an STD clinic in Paris. *Sex Transm Dis* 2010;37:153-8.
26. Risbud A, Chan-Tack K, Gadkari D, et al. The etiology of genital ulcer disease by multiplex polymerase chain reaction and relationship to HIV infection among patients attending sexually transmitted disease clinics in Pune, India. *Sex Transm Dis* 1999;25:55-62.
27. O'Farrell N. Soap and water prophylaxis for limiting genital ulcer disease and HIV-1 infection in men in sub-Saharan Africa. *Genitourin Med* 1993;69:297-300.

28. Kalichman SC, Simbayi LC, Cain D, Cherry C, Jooste S. Coital bleeding and HIV risks among men and women in Cape Town, South Africa. *Sex Transm Dis* 2006;33:551-7.
29. Mehta SD, Krieger JN, Agot K, Moses S, Ndinya-Achola JO, Parker CB, Bailey RC. Circumcision and reduced risk of self-reported coital injuries: Results from a randomized controlled trial in Kisumu, Kenya. *J Urol* 2010;184:203-9.
30. Mallon E, Hawkins D, Dinneen M, Francis N, Fearfield L, Newson R, Bunker C. Circumcision and genital dermatoses. *Arch Dermatol* 2000;136:350-354.
31. Mehta SD, Maclean IW, Bailey RC, Moses S, Gillevet P, Spear GT. Microbial diversity of genital ulcer disease in men enrolled in a randomized trial of male circumcision in Kisumu, Kenya. 19th Annual Meeting of the International Society for Sexually Transmitted Disease Research, Québec City, Canada, July 10 to 13, 2011. Abstract number P3-S5.01. doi:10.1136/sextrans-2011-050108.468.
32. de Sousa JD, Müller V, Lemey P, Vandamme AM. High GUD incidence in the early 20 century created a particularly permissive time window for the origin and initial spread of epidemic HIV strains. *PLoS One* 2010;5:e9936.
33. Paz-Bailey G, Sternberg M, Puren AJ, Steele L, Lewis DA. Determinants of HIV type 1 shedding from genital ulcers among men in South Africa. *Clin Infect Dis* 2010;50:1060-7.
34. FHI and the Centre for the AIDS Programme of Research in South Africa. *Factsheet: CAPRISA 004 Trial and the impact of tenofovir gel on herpes simplex virus type-2 infections.* Research Triangle Park, NC USA, July 2010. Accessed from http://www.usaid.gov/our_work/global_health/aids/TechAreas/research/caprisa_hsv2.pdf, on August 18, 2011.

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 & 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 etiology	30 (50.8)	18 (36.7)

HSV-2 = *Herpes simplex* virus type 2.
GUD = Genital ulcer disease (detected by physical examination or by patient report)

Table 2: Incident HSV-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*			
Circumcised	86/1493.5	5.8	0.94 [0.70,1.25]
Uncircumcised	100/1628.5	6.1	p=0.655
Incident syphilis+			
Circumcised	7/1897.5	0.4	1.23 [0.41,3.65]
Uncircumcised	6/1976.0	0.3	p=0.714
GUD post enrollment			
Circumcised	51/1912.0	2.7	0.52 [0.37,0.73]
Uncircumcised	101/1950.0	5.2	p<0.001
GUD post enrollment among HSV-2 positives at enrollment			
Circumcised	26/ 524.5	5.0	0.57 [0.35,0.95]
Uncircumcised	40/ 472.0	8.5	p=0.029
GUD post enrollment among HSV-2 negative 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 post enrollment among HSV-2 positive during follow-up			
Circumcised	8/119.0	6.7	0.33 [0.15,0.75]
Uncircumcised	25/125.5	19.9	p=0.008

* HSV-2 incidence is computed among participants who were HSV-2 seronegative at enrollment.

+ Syphilis incidence is computed among participants without syphilis detected prior to enrollment.

Cases are attributed to circumcised or uncircumcised status at the time detected positive. Person-years in circumcised and uncircumcised states are reported, where 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.

Table 3. Location of Genital Ulcers on Exams Among Treatment Group Subsets*

A. Location of Genital Ulcers Prior to Randomization			
Location+	Circumcised - n (%)	Uncircumcised - n (%)	p-value
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	1,304	1,393	
B. Location of Genital Ulcers on Follow-up Exams			
Location+	Circumcised - n (%)	Uncircumcised - n (%)	p-value
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	1,255	1,331	

* 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.
+ Locations are not mutually exclusive; ulcers may occur in multiple locations

Table 4A: HIV Risk Ratios [95% CI] by HSV-2, Genital Ulcer Disease and Circumcision Status, Overall (N=2,749).

Covariate	Unadjusted	Adjusted for Circumcision Status	Adjusted for All Covariates Shown
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

Table 4B: HIV Risk Ratios [95% CI] by HSV-2, Genital Ulcer Disease and Circumcision Status, Among Men Who Were HSV-2 Negative at Baseline (N=2,021).

Covariate	Unadjusted	Adjusted for Circumcision Status	Adjusted for All Covariates Shown
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.

Table 5. Sequence of HSV-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)