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Jyotsna Ramachandran Date

Interaction of Genital Abnormalities and Risk of HIV Acquisition among Women in Sero-Discordant Couples in Lusaka, Zambia

By

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Master of Public Health

Epidemiology

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By

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B.A., Washington University in St. Louis, 2012

Faculty Thesis Advisor: Kristin Wall, Ph.D.

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Abstract

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By Jyotsna Ramachandran

Background: Previous studies primarily focus on the effect of sexually transmitted infections (STIs) in HIV at-risk persons on sexual HIV acquisition risk; little consideration has been given to other non-STI genital ulcerative and inflammatory (GUI) abnormalities, or to the GUIs of HIV+ sexual partners. Information on the effect of multiple concurrent GUIs in both the donor and recipient on HIV transmission risk is scarce. This study examines the role of multiple GUIs, measured in both sexual partners, on women's HIV acquisition risk.

Methods: HIV serodiscordant heterosexual couples enrolled through couples' voluntary HIV counseling and testing (CVCT) were followed longitudinally from 1994-2012 with censoring at ART initiation, death/separation within the couple, or loss to follow-up. This analysis is restricted to couples where the man was the HIV+ partner (M+F-) and incident HIV infections were genetically linked to the study partner. HIV-negative partners were re-tested every three months for incident HIV infection. Indicators of GUI measured at study visits in both partners included: discharge, inguinal adenopathy (IA), bacterial vaginosis (BV), Candida, Trichomoniasis, ulcer, erosion/friability of the cervix/vagina, and smegma. Multivariable Cox models evaluated associations between GUIs and time to HIV infection, adjusting for age, viral load, and time enrolled.

Results: 207 infections occurred among 1,348 M+F- couples over 2,756 couple-years. Risk of infection in women was associated with women's: discharge (adjusted hazard ratio, aHR:1.97), Candida (aHR:1.72), ulcer (aHR:2.47), erosion/friability of the cervix/vagina (aHR:2.45), IA (aHR:2.13); and men's discharge (aHR:4.02), ulcer (aHR:1.55), IA (aHR:2.02), and smegma (aHR:1.81). Pairs of exposures significantly associated with HIV infection (versus having neither GUI) that had a synergistic effect on transmission risk were: BV and discharge (aHR:2.59) and Candida and discharge (aHR:3.21) (women); discharge and IA (aHR:4.98) (men); men and women with ulcers (aHR:4.78), and female ulcer with male smegma (aHR:5.34).

Conclusion: Increased routine screening/treatment of non-STI GUIs associated with HIV transmission is needed in both partners. Simultaneous screening/treatment for multiple GUIs is warranted since there is often clustering of these exposures, and many pairs act synergistically. Exploring low cost home-hygiene based methods of screening/treatment may be beneficial.

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Chapter I. Background/Literature Review

HIV Epidemic in Sub-Saharan Africa

The HIV epidemic continues to be a serious concern in sub-Saharan Africa. As of 2013, it was reported that 24.7 million people in the region were living with HIV. There were estimated to be 1.5 million new cases of HIV in sub-Saharan Africa in 2013, and this region accounts for approximately 70% of the new HIV infections globally(1). The majority of new HIV infections in sub-Saharan Africa occur from heterosexual transmission(2-4). The high rate of HIV transmission among heterosexual couples has been attributed to the large numbers of sero-discordant heterosexual couples living in Africa with the term "sero-discordant couple" defined as a couple where one partner is HIV positive and the other partner is HIV negative(3). Furthermore it has been determined that approximately two thirds of incident HIV infections in sub-Saharan Africa occur among stable couples(5), with approximately 30% of incident HIV infections occurring among stable sero-discordant couples(6), and a majority of heterosexual HIV transmissions in urban Zambia occurring within marriages(3). These findings suggest that stable sero-discordant couples comprise a target demographic for intervention to reduce the HIV epidemic in this region.

While research studies conducted in Rwanda and Zambia have found couples' voluntary HIV testing and counseling (CVCT) to be effective in reducing risk of transmission between heterosexual, sero-discordant partners(2), it is equally

important to continue identifying predictors and co-factors in HIV transmission given that CVCT is not widely available nor does it completely eliminate transmission and acquisition risk(2, 7). Similarly, antiretroviral therapy (ART), while effective in reducing risk of HIV transmission in sero-discordant couples, is not widely available or adhered to(8, 9). Identifying additional co-factors in HIV transmission and acquisition will allow us to better target screenings and interventions in at-risk populations, and will help reduce HIV incidence, particularly in high burden areas such as sub-Saharan Africa.

STIs/Genital Abnormalities and HIV Incidence

Several studies have found an appreciable association between certain sexually transmitted infections (STIs) and other genital abnormalities and increased transmission and acquisition of HIV(10). Of note, Herpes Simplex Virus 2 (HSV-2) has been found to be a predictor of increased risk of HIV transmission and acquisition among populations in sub-Saharan Africa(4, 10). Venkatesh et. al. (2011) conducted a nested case-control study among 4948 enrolled HIV negative women in South Africa. In the study, 309 incident HIV cases and 927 controls were analyzed, and they found that incidence of HIV was significantly higher among women with prevalent or incident HSV-2 infection, and it was also higher among women with incident Neisseria gonorrhea infection. In their study, the population attributable fraction for prevalent HSV-2 was 29%, for incident HSV-2 was 2.1% and for incident N. gonorrhea was 4.1%, and they propose incident bacterial and viral STIs to be risk

factors for HIV incidence (4). In a review, Ward et. al. (2010) discuss a cohort study of 4439 women in Zimbabwe and Uganda, followed every 3 months for up to 2 years, and found the population attributable risk percentage (PAR%) for prevalent HSV-2 to be 50.4% and incident HSV-2 was 7.9%(10). The same study also estimated a PAR% of 5.3 for gonorrhea and 17.2 for bacterial vaginosis(10).

Other studies have found significant associations between bacterial vaginosis (BV) infection and an increased risk of HIV transmission (11). In a prospective cohort study of n=1964 Kenyan female sex workers that spanned from 1993 to 2012, Masese et. al. (2015) found significant associations between BV and incident HIV; however, the PAR% for trichomoniasis, gonorrhea and genital ulcer disease (GUD) were under 3% in this study. Using Cox regression models adjusted for age, workplace, sexual risk behavior, hormonal contraceptive use and other STIs, they found an adjusted hazard ratio of 1.86 (95% CI: 1.40, 2.47) for BV, and the PAR was 15.1%(11). Along with HSV-2, BV was identified as having one of the highest attributable risks (11). In a cohort study of n=1037 Ugandan female sex workers and women working in bars, which looked at factors associated with BV infection, Francis et. al. (2015) found prevalent BV to be associated with both HSV-2 and prevalent HIV(12). Because the association was with prevalent HIV, it is not possible to examine BV as a risk factor for incident HIV in this study (12). However, as a way of explaining a possible underlying biological mechanism, it has been proposed that the lactobacillus bacteria present during BV infection may increase risk of other STI infections, including

incident HIV infection, by reducing vaginal acidity, which usually acts to protect against these bacterial and viral infections(12).

In a separate study conducted among n=435 women in Durban, South Africa, BV was found to be positively associated with incident Trichomonas vaginalis infection and incident Chlamydia trachomatis infection(13), suggesting that there is clustering of STIs occurring in these populations. In a prospective cohort study previously described in the review by Ward et. al. (2010), van de Wijgert et. al. (2009) investigated the temporal relationship between reproductive tract infections and HIV incidence among n=4439 HIV uninfected women in Zimbabwe and Uganda(14). They found HIV acquisition to be associated with incident HSV-2 (HR 5.35, 95% CI: 3.06, 9.36), incident Neisseria gonorrhoeae (HR 5.46, 95% CI: 3.41-8.75), and with BV infection (HR: 2.12, 95% CI: 1.50, 3.01) after adjusting for condom use, age, women's risk behavior, partner's risk behavior, and other STIs when looking at a specific genital abnormality's relationship with HIV infection (14). Association between Trichomonis vaginalis and incident HIV was statistically insignificant in this study (aHR: 1.20, 95% CI: 0.57, 2.51)(14). A study conducted among 646 Ugandan female sex workers by Vandepitte et. al, found Trichomonas vaginalis (aHR: 2.26, 95% CI: 1.03, 4.93) to be significantly associated with HIV acquisition after adjusting for age, calendar time, age at first intercourse, alcohol use, consistency of condom use with paying customers, number of lifetime partners and number of paying customers in the past 3 months(15). Trichomoniasis infection was identified prior to the clinic visit where the woman was found to be HIV positive suggesting a temporal

relationship (15). Overall, the literature heavily focuses on the association between HSV-2 and incident HIV infection and BV and incident HIV infection among African women. The true magnitude of the association between other STIs such as syphilis, trichomoniasis, chlamydia, and gonorrhea, and incident HIV infection is unclear, and associations between Candida infection and HIV transmission or acquisition remain largely unstudied.

Genital Ulceration and Inflammation

More broadly, genital ulcer disease (GUD) and genital inflammation, the primary signs/symptoms associated with genital abnormalities and STIs, have been found to be associated with an increased risk of HIV transmission and acquisition. HSV-2 is considered a leading cause of genital ulcer disease(16); however, there are several etiologies responsible for genital ulcers. In a study of 387 men who are HIV positive, Paz-Bailey et. al. (2010) found that men who also have genital ulcer disease demonstrate increased HIV viral shedding (16), which potentially increases risk of transmission to their sero-negative female partners. Genital ulcers in the female genital tract serve to disrupt the epithelium, increasing risk of HIV infection in HIV negative females(17). In a previous study of a cohort of 2,949 sero-discordant couples in Lusaka, Zambia, a composite measure of genital ulceration in the recipient was associated with HIV transmission, and a composite measure of genital inflammation in the donor or recipient was associated with HIV infection(18). In M+F- couples, the aHR for female inflammation was 2.25 (95%CI: 1.54, 3.29), male inflammation aHR:

2.97 (95%CI: 1.94, 4.57), and female ulceration aHR was 2.41 (95%CI: 1.59, 3.67). In M-F+ couples, the aHR for female inflammation was 2.96 (95%CI: 1.89, 4.62), male inflammation was aHR: 3.36 (95%CI: 2.03, 5.56) and male ulceration aHR was 2.73 (95%CI: 1.71, 4.37) after adjusting for age, viral load of the positive partner, and time since enrollment in CVCT(18).

A nested case control study of 116 women from an underlying prospective cohort of 889 HIV uninfected women in South Africa found a significantly higher risk of HIV infection among women with genital inflammation with an unadjusted odds ratio of 3.2 (95% CI 1.3-7.9)(19). Literature is sparse regarding the association between inflammation caused by inguinal adenopathy and risk of HIV transmission or acquisition. Inguinal adenopathy is often reported as a symptom of HIV infection(20), but it has not been investigated extensively as a risk factor. Research is also sparse regarding male hygiene as related to genital inflammation, and its role as a potential risk factor for HIV transmission. The presence of smegma under the male foreskin, an accumulation of white blood cells, is sometimes a symptom of male genital inflammation, or sometimes a cause of male genital inflammation, but its role in HIV risk is presently unclear(21).

Biological Mechanisms for Increased HIV Transmission Risk

HIV transmission primarily occurs during sexual intercourse at mucosal surfaces in the genital tract, and genital tract mucosa is reported to be the major site of transmission and acquisition particularly among HIV negative women (22), with

CD4+ T cells acting as the main target for HIV infection (23). The epithelium covering the vagina and ectocervix contains CD4+ and CD8+ T-cells and Langerhans cells, which have HIV receptors making it vulnerable to infection(23). During sexual intercourse, microscopic tears in the genital epithelium may result and there is potentially an increased cytokine response to semen, both of which increase a seronegative woman's risk of HIV acquisition. It has been hypothesized that STIs pose a disruption to the genital tract mucosa and genital tract microbiota, which may also facilitate HIV transmission and acquisition(23). For example, bacterial vaginosis and trichomonas vaginalis are said to alter the cervico-vaginal immune and inflammatory response increasing the risk of HIV acquisition by two to three fold(17). Ulcerative STIs are thought to increase risk of HIV acquisition by compromising the lower genital tract pseudosquamous epithelium and exposing lamina propria target cells for infection(17).

Alternate studies suggest that inflammatory cytokines in the genital tract may stimulate viral replication and recruit target cells, which in turn promotes increases in HIV shedding. These cytokines may also be produced due to STI infection (22). Masson et. al (2015). found genital inflammation is not limited to symptomatic STIs in a study of 227 women in Durban, South Africa. Women with asymptomatic STIs, such as chlamydia and gonorrhea, were also found to have high concentrations of genital tract cytokines(24, 25). Furthermore, a study by Mlisana et. al. (2012) found, among 242 female participants in South Africa, only 12.3% of women who had a laboratory diagnosed, discharge causing STI actually presented clinically with vaginal discharge(26). This finding highlights the importance of screening for, more

accurately diagnosing, and treating both symptomatic and asymptomatic STIs as risk factors for HIV transmission, especially in high burden areas where genital inflammation from untreated STIs could increase the risk of HIV infection(24, 25).

Multiplicative Interaction Between STI and Genital Abnormality Predictors

The majority of the current literature aims to address if and how STIs and other genital abnormalities increase the risk of HIV acquisition, however another point of consideration is if there is multiplicative or additive interaction between STIs in increasing risk of HIV acquisition and transmission. While some studies have suggested this may be the case, there is not an abundance of research in this area. For example, recent studies have found that among HIV positive men with ulcers, those with Trichomonas vaginalis infections had higher ulcer viral loads (where an ulcer viral load is the HIV RNA viral load determined by swabbing genital ulcers) when compared to those not infected with Trichomonas vaginalis(16). Since different STIs affect the immune system and genital tract differently, it is possible that women with multiple STIs or symptoms of genital ulceration and/or inflammation, or women with STIs who also have partners with concurrent STIs or genital ulceration and/or inflammation may be at much higher risk of HIV acquisition. This is of particular importance, especially in low resource settings, because it allows prioritization for targeting limited resources to populations that may be of highest risk.

Limitations in the Current Research

The current body of research often fails to account for the study of partners and couples in terms of risk factors for HIV transmission. Although this is often a limitation of study design, it is an important factor to consider when discussing HIV transmission risk, especially among heterosexual men and women who are in stable sero-discordant couples. Missing this information could be biasing estimates in previous studies because it does not allow the adjustment for potential confounding contributed by partner behavior or partner's biological risk factors. This analysis aims to address that gap in the research. Additionally, the longitudinal prospective cohort design of our study population allows us to assess temporality when looking at STI infection, genital ulceration and genital inflammation in relation to incident HIV infection, and data on multiple GUIs in both study partners allows us to consider GUI-GUI interactions.

CHAPTER II. MANUSCRIPT

Interaction of Genital Abnormalities and Risk of HIV Acquisition among Women in Sero-Discordant Couples in Lusaka, Zambia

Jyotsna Ramachandran

Abstract:

Background: Previous studies primarily focus on the effect of sexually transmitted infections (STIs) in HIV at-risk persons on sexual HIV acquisition risk; little consideration has been given to other non-STI genital ulcerative and inflammatory (GUI) abnormalities, or to the GUIs of HIV+ sexual partners. Information on the effect of multiple concurrent GUIs in both the donor and recipient on HIV transmission risk is scarce. This study examines the role of multiple GUIs, measured in both sexual partners, on women's HIV acquisition risk.

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Results: 207 infections occurred among 1,348 M+F- couples over 2,756 couple-years. Risk of infection in women was associated with women's: discharge (adjusted hazard ratio, aHR:1.97), Candida (aHR:1.72), ulcer (aHR:2.47), erosion/friability of the cervix/vagina (aHR:2.45), IA (aHR:2.13); and men's discharge (aHR:4.02), ulcer (aHR:1.55), IA (aHR:2.02), and smegma (aHR:1.81). Pairs of exposures significantly associated with HIV infection (versus having neither GUI) that had a synergistic effect on transmission risk were: BV and discharge (aHR:2.59) and Candida and discharge (aHR:3.21) (women); discharge and IA (aHR:4.98) (men); men and women with ulcers (aHR:4.78), and female ulcer with male smegma (aHR:5.34).

Conclusion: Increased routine screening/treatment of non-STI GUIs associated with HIV transmission is needed in both partners. Simultaneous screening/treatment for multiple GUIs is warranted since there is often clustering of these exposures, and many pairs act synergistically. Exploring low cost home-hygiene based methods of screening/treatment may be beneficial.

Introduction:

As of 2013, it was reported that 24.7 million people in Sub-Saharan Africa were living with HIV, and there were estimated to be 1.5 million new cases of HIV in 2013; this region accounts for approximately 70% of new HIV infections globally(1). The majority of new HIV infections in sub-Saharan Africa occur from heterosexual transmission among stable sero-discordant couples(2-6). These findings suggest that interventions among this demographic will be integral to reducing the HIV epidemic in this region.

Identifying predictors in HIV transmission is imperative for reducing HIV incidence by enabling improved screening and treatment interventions targeted towards at-risk populations, particularly in high burden, low resource areas. Several studies have found an appreciable association between sexually transmitted infections (STIs) and genital abnormalities, such as HSV-2, bacterial vaginosis, Trichomoniasis and Neisseria gonorrhea, and increased acquisition of HIV in women(4, 10-12, 14, 15, 27). More broadly, genital ulcer disease (GUD) and genital inflammation, the primary signs/symptoms associated with STIs, have been found to be associated with an increased HIV transmission and acquisition risk(16-18, 24).

However, few studies have looked at other genital abnormalities such as Candida sp infection, inguinal adenopathy, foreskin smegma, and erosion and friability of the cervix or vagina, which are not sexually transmitted, in relation to risk of HIV acquisition in HIV negative women. Additionally, the current body of research often neglects to account for genital abnormalities in partners and their role in increasing transmission risk

when assessing risk of HIV infection. Missing this information could be biasing risk estimates in previous studies by essentially ignoring the positive partner's biological risk factors. Furthermore, very little work has been done to explore and quantify interaction between multiple genital abnormalities and the impact of this interaction on HIV infection risk; this shortage is usually due to study design limitations.

Our study explores the risk associated with several non-sexually transmitted genital abnormalities along with some sexually transmitted infections and HIV transmission and acquisition. We also explore interactions between multiple genital abnormalities either in a given individual or for a couple, which is important due to clustering of genital abnormalities (13). The findings from our study contribute to creating a more comprehensive picture of predictors of HIV transmission in a high burden setting. The results can also be used to enhance couples counseling services, integrate screening for several genital abnormalities, and inform simple and efficient treatment interventions that would play a role in reducing incident HIV infections.

Methods:

Study Site and Population:

Married or cohabiting couples living in Lusaka, Zambia were recruited through couples voluntary HIV counseling and testing (CVCT) services provided by the Emory-based Rwanda Zambia HIV Research Group (RZHRG) into a longitudinal cohort with open enrollment from 1994-2012. Couples were recruited for counseling either spontaneously or through an invitation from a community promoter. The couples counseling services

provided couples with rapid HIV testing with mutual disclosure of results, and follow up HIV-counseling, which aimed to reduce HIV transmission risk over the course of enrollment.

The longitudinal cohort includes heterosexual HIV sero-discordant couples where one partner is HIV positive, and the other partner is HIV negative, i.e M+F- or M-F+. Baseline covariates were obtained for the couples at study entry, and follow up data was collected at follow-up study visits. For this analysis, we will be focusing on the M+F- sero-discordant couples only. The study population included 1,348 M+F- couples, of which, 1,141 couples were non-transmitters (the negative partner did not contract HIV during the study period), and 207 couples were transmitters (the negative partner contracted HIV during the study period). All transmissions in our study population were linked transmissions, where the HIV negative partner was infected by their study partner. Couples with transmissions where the HIV negative partners were likely infected by outside sexual partners were excluded from this analysis.

Outcome of Interest:

Time to HIV sero-conversion in the female HIV-negative partner is the outcome of interest. HIV status was determined by the use of rapid serologic tests, which were conducted at one to three-month intervals during follow-up visits. Linked versus non-linked transmission was determined by comparing conserved PCR-amplified nucleotide sequences from each member of the couple to confirm if the viral strain was genetically

linked to the study partner.

Exposures of Interest:

Exposures of interest in the study include: Trichomonas vaginalis infection, Candida sp infection, bacterial vaginosis infection, genital discharge, inguinal adenopathy, genital ulceration, erosion/friability of the cervix/vagina, and incident syphilis infection, in women. In men, the exposures of interest include inguinal adenopathy, genital ulceration, incident syphilis infection, personal hygiene indicated by presence of smegma under the foreskin, circumcision status, and genital discharge.

Baseline Covariates:

Demographic covariates were collected at the initial baseline visit with information about age of each study partner, number of years living together, Nyanja literacy, alcohol use, family planning as evaluated based on number of previous pregnancies, whether or not the female was pregnant at baseline, and the fertility intentions of the male and female study partners based on self-report. Information about the couples' sexual history was also collected including number of lifetime partners, number of partners in the last year, circumcision status of the male partner, and whether or not the participant had an STI in the past year, also using self-report. Clinical characteristics obtained at baseline included the HIV stage of the positive partner and the plasma viral load of the positive partner. Viral load of the positive partner was recorded beginning in 1999.

Time Varying Covariates:

Time varying covariates were collected for each partner during follow up study visits. Laboratory diagnosis or symptom based treatment was used to identify cases of Trichomonas vaginalis infection, Candida sp infection, and bacterial vaginosis infection. Self-reported or clinical diagnoses were used to identify cases of vaginal discharge or urethral discharge, inguinal adenopathy, genital ulceration, and erosion or friability of the cervix/vagina. Positive rapid plasma reagin serology tests were used to confirm syphilis infection. For men, presence of smegma under the foreskin was determined based on physical exam and circumcision during follow-up was confirmed using self-report and clinical confirmation. Time varying covariates were collected for all time periods of the study, except for inguinal adenopathy for males and females, presence of smegma under the foreskin, and erosion and friability of the cervix/vagina, which were only collected consistently until 2002.

Model Building Methods:

SAS 9.4 was used for all data analysis. Couples were censored if either partner was lost to follow-up, either partner died, the couple separated, or if the HIV-positive partner started antiretroviral treatment.

Cox extended-Proportional Hazards models were used to conduct survival analysis and assess crude associations (hazard ratios) between each genital abnormality and time to incident HIV infection. Crude hazard ratios were also obtained for having two genital abnormalities concurrently (versus having neither) to assess multiplicative interaction between genital abnormalities and risk of HIV infection.

Adjusted Cox models were adjusted for viral load of the positive partner, woman's age, and if it had been at least three months since the couple entered CVCT. Inclusion of these covariates as potential confounders was determined based on previous analyses of this dataset(18). Additionally, models were adjusted for genital inflammation if the exposure(s) of interest was an ulcerative exposure, or for genital ulceration if the exposure(s) of interest was inflammatory. When looking at select pairs of genital abnormalities, models were not adjusted for genital ulceration or inflammation if the pair included one ulcerative exposure and one inflammatory exposure.

All time-independent covariates included in the model met the proportional hazards assumption demonstrated by the values of the Shoenfeld's Residuals. Collinearity of the individual genital ulceration and inflammation components was assessed before including the variables in multivariable models. Collinearity was determined using cut-points of condition indices greater than 10 and variance decomposition proportions greater than 0.5; however, no exposure variable was found to exceed these cut-points.

Expected hazard ratios for people with select pairs of genital abnormalities (versus having neither genital abnormality) were calculated as a product of the adjusted hazard ratios obtained for having each genital abnormality alone. If the expected hazard ratio differed from the observed adjusted hazard ratio for those having both genital abnormalities by 10% or more, the exposure pair was deemed to show a synergistic (if observed hazards ratio was greater than expected) or antagonistic (if observed hazards ratio was less than expected) effect.

Population attributable fractions were calculated based on the adjusted hazard ratios obtained from the adjusted Cox Proportional Hazards models using the following formula(28-30):

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

In the above formula, p is the proportion of cases that are exposed.

Results:

1,348 M+F- couples were followed and collectively contributed 2,756 couple years (CY) to the study. Among these sero-discordant couples, there were 207 genetically linked HIV transmissions over the course of follow up.

Genital Abnormality Exposure Distributions and Crude Associations (Table 1):

Many of the time-varying genital abnormality exposures were significantly associated with the outcome of interest, incident HIV infection in women, with a p-value<0.05 (Table 1). Exposures with significant associations with HIV infection in crude analysis included, for women, genital discharge, Candida, Trichomoniasis, genital ulcer, erosion/friability of the cervix/vagina, and inguinal adenopathy. For men, significant exposures included genital discharge, active ulcer, inguinal adenopathy, and smegma.

After adjusting for woman's age, HIV viral load of the positive partner at baseline, being in the 0-3 month follow up interval since enrollment versus greater than 3 months, and genital inflammation (if looking at an ulcerative exposure) or genital ulceration (if looking at an inflammatory exposure), the following exposures remained significantly associated with the outcome for women: genital discharge (aHR: 1.97, 95%CI: 1.26, 3.07), Candida (aHR: 1.72 95% CI: 1.08, 2.76), genital ulcer (aHR: 2.47, 95% CI: 1.35, 4.53), erosion/friability of the cervix or vagina (aHR: 2.45, 95% CI: 1.19, 5.05), and inguinal adenopathy (aHR: 2.13, 95% CI: 1.25, 3.62). For men, significant exposures again included genital discharge (aHR: 4.02, 95% CI: 2.26, 7.16), genital ulcer (aHR: 1.55, 95% CI: 1.09, 2.19), inguinal adenopathy (aHR: 2.02, 95%CI: 1.10, 3.70), and the male hygiene variable, smegma (aHR: 1.81, 95% CI: 1.03, 3.21).

Distributions of Concurrent Genital Abnormality Exposures (Table 2):

Table 2 shows distributions for select pairs of genital abnormalities and their crude association with HIV infection. Significant associations with the outcome were observed for several pairs of exposures (p-value <0.05).

In women, pairs of exposures (i.e., having both exposures versus neither) statistically significantly associated with the outcome were: BV and genital discharge, Candida and genital discharge, Trichomoniasis and genital discharge, BV and inguinal adenopathy, and genital discharge and inguinal adenopathy. In men, significant pairs of exposures include: genital discharge and ulcer, genital discharge and inguinal adenopathy, and smegma and inguinal adenopathy. When looking at exposures in both partners, men and women who both had active ulcers, and female ulcer with male smegma were significantly associated with HIV infection.

Exposure pairs that were not found to be significantly associated with HIV infection were Candida and Trichomoniasis, erosion/friability of the cervix/vagina and inguinal adenopathy in women, inguinal adenopathy and ulcer in women, ulcer and circumcision in men, and genital discharge and inguinal adenopathy in men.

Multiplicative Interaction, Adjusted Associations (Table 3):

Table 3 shows the adjusted hazard ratios (aHR) for the combinations of genital abnormality exposures after adjusting for woman's age, viral load of the positive partner, time enrolled in CVCT, and genital inflammation (if looking at an ulcerative exposure) or

genital ulceration (if looking at an inflammatory exposure). The expected combined effects were calculated for each pair of exposures and compared to the observed effects to assess for multiplicative interaction.

Genital abnormality exposures which had a synergistic effect in combination were: BV and discharge (aHR:2.59, 95% CI: 1.08,6.19), and Candida and discharge (aHR: 3.21, 95% CI: 1.60,6.45) in women. In men: discharge and IA (aHR:4.98, 95% CI: 2.23, 11.08). In men and women: male and female ulcers (aHR: 4.78, 95% CI: 2.00, 11.44), and female ulcer with male smegma (aHR:5.34, 95% CI: 1.87,15.23).

The remaining exposure pairs showed an antagonistic effect in combination.

Genital Abnormality Exposures, Population Attributable Fractions (Table 4):

Population attributable fractions (PAF) for individual genital abnormalities, and for having a combination of two genital abnormalities are reported in Table 4. In women, the PAF for women's genital discharge was 8%, and it was 6% for Candida, 6% for erosion or friability of the cervix or vagina, and 14% for inguinal adenopathy. In men, the PAF was 6% for male genital discharge, 10% for men's ulcer, 42% for men's inguinal adenopathy, and 7% for presence of smegma. When examining combined effects of two genital abnormality exposures, the PAF for bacterial vaginosis and discharge in women is 2%, for Candida and discharge in women, it is 4%, for discharge and inguinal adenopathy in women, it is 5%, for discharge and inguinal adenopathy in men, it was 6%, and for

inguinal adenopathy and smegma in men, it was 8%. For male and female active ulcers, the PAF was 3%.

Discussion:

In sub-Saharan Africa, where HIV burden and incidence of HIV infection remain high, particularly among stable, heterosexual couples(1-6), it is critical to identify predictors of HIV transmission and acquisition to better target screening and treatment interventions, especially in low-resource settings.

Among M+F- sero-discordant couples in Lusaka, Zambia, genital discharge, Candida infection, genital ulcer, erosion/friability of the cervix or vagina, and inguinal adenopathy (IA) in women, as well as genital discharge, genital ulcer, inguinal adenopathy (IA), and smegma in men, were found to be significant predictors of HIV infection. IA in women had a PAF of 14%, genital ulcer in men had a PAF of 10%, and IA in men had a PAF of 42%. These findings expand upon the growing literature about STIs and genital ulcers increasing risk of HIV infection(4, 10-12, 14-18, 24, 27), by investigating non-sexually transmitted genital abnormalities. Furthermore they provide an evidence base for future interventions since Candida, genital ulcer, genital discharge, inguinal adenopathy and smegma are treatable if diagnosed. Biological mechanisms that could explain the association between these genital abnormalities and increased HIV infection risk include: disruptions in the genital mucosa from ulceration, exposure of the lamina propria target cells for infection due to a compromised genital tract epithelium, changes to the genital

microbiome due to bacterial or viral infection, which facilitates HIV infection, and inflammatory cytokines in the genital tract that may stimulate viral replication and recruit target cells, which in turn promotes increases in HIV shedding(17, 22, 31).

Bacterial vaginosis, Trichomoniasis, and both male and female syphilis were not found to be significant predictors of HIV acquisition in this population, which contradicts findings in previous studies of African populations(11, 14, 15). However, BV with concurrent genital discharge was a statistically significant predictor in our study. We hypothesize that controlling for other risk factors in both partners when examining the effect of each genital abnormality exposure may have contributed to the differences observed, because several previous studies have looked at women or men independent of their partners. Furthermore, it may be the symptoms of ulceration or inflammation in the donor or recipient rather than the infection itself that is associated with increased risk of HIV acquisition, which could explain the observed results.

Very few studies have considered the effect of an individual having multiple genital abnormalities concurrently or for both partners having genital abnormalities concurrently and how this affects the risk of HIV infection. Our study is innovative in that it examined predictors of HIV infection at the couple level and investigated interactions between genital abnormalities.

When looking at select pairs of genital abnormalities, pairs of exposures that had a synergistic effect were: BV in women with female genital discharge, Candida in women

with female genital discharge, male discharge with male inguinal adenopathy (IA), concurrent male and female genital ulcers, and female ulcer with male smegma. Among the synergistic exposure pairs, the hazard observed among women with both BV and discharge is approximately 40% greater than the expected hazard among women with both exposures, and 40% greater than those with genital discharge alone; among women with Candida and discharge, the hazard observed was approximately 20% greater than expected. In men, those with discharge and IA had partners with 44% greater hazard of HIV infection compared to expected based on the hazards of each exposure alone, and among couples where both partners had genital ulcers, there was a 64% greater observed hazard. The increased risk observed supports the need to provide screening for several genital abnormalities within the same treatment facility during a patient visit. These findings also support the need to screen and treat both partners for genital abnormalities. Couples in counseling services should be educated about the increased risk of multiple exposures, and how to identify potential genital abnormalities, particularly genital ulcers and discharge, so that they can be vigilant about seeking care and self-reporting symptoms to receive treatment. Additionally, as most GUIs identified were non-STIs, exploring low cost, home-hygiene based intervention methods may be beneficial. Educating men on the importance of hygiene and cleaning the foreskin to eliminate smegma, and raising awareness about the role vaginal douching may play in potentially increasing women's risk of BV infection(32) are relatively cheap interventions that could decrease prevalence of these genital abnormalities in at-risk populations.

Exposure pairs that showed an antagonistic effect were erosion/friability of the cervix or vagina with IA in women, IA with genital ulcer in women, BV and IA in women, and discharge and IA in women, IA and smegma in men, and discharge and ulcer in men. Many of the antagonistic pairings include either male or female IA, which we hypothesize could suggest that those with IA may be seeking care earlier due to discomfort, which could be producing the antagonistic effect observed in the results. Exposure pairs that were not significantly associated with HIV infection include Candida with Trichomoniasis, erosion/friability of the cervix/vagina with IA in women, IA with genital ulcer in women, genital ulcer and circumcision in men, and discharge and IA in men. However, in many of these pairs, each exposure alone was found to be significantly associated with the outcome, and insignificant p-values could be due to small sample size. Further studies are needed to determine the true effect of these exposure pairs on HIV infection risk.

Strengths and Limitations:

Limitations of the study include missing values with higher percentages of missing values for certain genital abnormalities such as male discharge, male genital ulcer, and male syphilis, which may affect generalizability of the study results. Analysis of this study cohort found loss to follow-up was associated with residence further from the study clinic, younger age, and woman's age ≤17 upon first intercourse among M+F-couples(7). Certain GUI components were collected using different methods during different periods of the study or were only collected consistently until 2002, limiting the

available data. However, since there is no differential missingness of data due to changes in collection method by the outcome of interest, we would expect this possible limitation to bias our results toward the null. There is potentially misclassification bias due to self-reporting diagnoses of some genital abnormalities and potential clinical misclassification, which, if differential by the outcome, could bias our results in an unknown direction. There is also potential for selection bias, which limits generalizability because these couples were recruited through couples counseling services, and their risk of HIV infection could differ from couples that did not seek couples counseling or volunteer to participate in the study.

Strengths of the study include a longitudinal, prospective cohort with follow up data at one to three month intervals and information on both partners better allowing us to assess temporality and hypothesize causal relationships between the observed genital abnormalities and increased HIV acquisition. This level of observational data is particularly important since hypotheses related to HIV incidence cannot be examined using clinical trial methods, and extensive observational data is necessary to examine the relationship. Data for both partners within the sero-discordant couple also allowed us to more fully consider risk factors for increased HIV transmission and acquisition. Clinical diagnosis of HIV status and clinical lab results for several of the STI and genital abnormality diagnoses are also strengths of the study. Additionally, examination of several genital abnormality exposures over time allowed us to consider GUI-GUI interactions.

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Table 1. Distributions and associations between genital abnormality predictors and HIV infection among M+F-couples, Lusaka, Zambia

	Linked Transmission		Non-transmitting Intervals						
	N Events	Intervals	N Events	Intervals	Crude p-value	aHR*	95% CI		Adjusted p-value
Women									
Genital Discharge	33	206	636	13,140	<.0001	1.97	1.26	3.07	0.0029
Bacterial Vaginosis (BV)	18	207	570	13,411	0.2544	1.17	0.68	2.02	0.5769
Candida	29	207	689	13,411	0.0032	1.72	1.08	2.76	0.0236
Trichomoniasis	16	207	403	13,364	0.0032	1.50	0.81	2.77	0.1960
Ulcer	16	206	251	13,156	<.0001	2.47	1.35	4.53	0.0034
Incident Syphilis (RPR)	8	207	210	13,408	0.0904	1.69	0.81	3.54	0.1650
Erosion/friability of cervix/vagina[a]	10	94	177	3,789	0.0050	2.45	1.19	5.05	0.0151
Inguinal adenopathy (IA)[a]	25	94	381	3,797	<.0001	2.13	1.25	3.62	0.0054
Men									
Genital Discharge	15	194	188	11,154	<.0001	4.02	2.26	7.16	<.0001
Ulcer	53	191	1,744	10,966	0.0004	1.55	1.09	2.19	0.0135
Incident Syphilis (RPR)	5	195	324	11,141	0.4332	0.74	0.30	1.82	0.5100
Circumcision	15	207	1,327	13,439	0.4591	0.90	0.52	1.56	0.7111
Inguinal adenopathy (IA)[a]	78	94	2,685	3,803	0.0105	2.02	1.10	3.70	0.0226
Smegma[a]	15	94	351	3,808	0.0275	1.81	1.03	3.21	0.0406

[a] collected until 2002

* adjusted for age, viral load of positive partner, time since enrollment in counseling, and inflammation if ulcerative exposure, ulceration if inflammatory exposure.

Table 2. Combinational distributions of genital abnormality exposures by HIV infection outcome, M+F-couples, Lusaka, Zambia

	Linked Transmission		Non-Transmitting Intervals		Crude p-value*
	N Events	% Intervals	N Events	% Intervals	
Women					
BV and Genital Discharge					
BV alone	10	5%	477	4%	0.8674
Genital discharge alone	25	12%	543	4%	<.0001
BV and genital discharge	8	4%	93	1%	0.0002
Neither BV or genital discharge	163	79%	12,027	92%	ref
Candida and Genital Discharge					
Candida alone	17	8%	530	4%	0.0493
Genital discharge alone	21	10%	477	4%	0.0001
Candida and genital discharge	12	6%	159	1%	<.0001
Neither Candida or genital discharge	156	76%	11,974	91%	ref
Trichomoniasis and Genital Discharge					
Trichomoniasis alone	11	5%	335	3%	0.0185
Genital discharge alone	28	14%	568	4%	<.0001
Trichomoniasis and genital discharge	5	2%	68	1%	0.0026
Neither Trichomoniasis or genital discharge	162	79%	12,141	93%	ref
Candida and Trichomoniasis					
Candida alone	27	13%	657	5%	0.0027
Trichomoniasis alone	14	7%	371	3%	0.0029
Candida and Trichomoniasis	2	1%	32	0.20%	0.1245
Neither Candida or Trichomoniasis	164	79%	12,304	92%	ref
Erosion/friability of the cervix/vagina and IA[a]					
Erosion Alone	8	9%	131	3%	0.0003
IA alone	23	24%	335	9%	<.0001
Erosion and IA	2	2%	46	1%	0.2831
Neither Erosion or IA	61	65%	3,280	86%	ref
IA and Ulcer[a]					
Ulcer alone	8	9%	120	3%	0.0057
IA alone	23	24%	337	9%	<.0001
Ulcer and IA	2	2%	44	1%	0.0676
Neither ulcer or IA	61	65%	3,297	87%	ref
BV and IA[a]					
BV Alone	12	13%	485	13%	0.3645
IA alone	20	21%	323	9%	<.0001
BV and IA	5	5%	58	2%	0.0057
Neither BV or IA	57	61%	2,931	77%	ref

Genital Discharge and IA[a]					
Genital discharge alone	14	15%	414	11%	0.0688
IA alone	16	17%	287	8%	0.0003
Genital discharge and IA	9	10%	94	2%	<.0001
Neither genital discharge or IA	55	58%	3,001	79%	ref
Men					
Ulcer and circumcision					
Circumcision alone	12	6%	1,200	11%	0.4079
Ulcer alone	50	26%	1,617	14%	0.0005
Circumcision and ulcer	3	2%	127	1%	0.5472
Neither circumcision or ulcer	127	66%	8,262	74%	ref
Genital Discharge and Ulcer					
Genital discharge alone	11	6%	110	1%	<.0001
Ulcer alone	49	26%	1,666	15%	0.0002
Genital Discharge and ulcer	4	2%	78	1%	0.0313
Neither genital discharge or ulcer	128	67%	9,114	83%	ref
Genital Discharge and IA[a]					
Genital discharge alone	1	1%	28	1%	0.3485
IA alone	71	76%	2,607	68%	0.0906
Genital discharge and IA	7	7%	78	2%	<.0001
Neither genital discharge or IA	15	16%	1,094	29%	ref
IA and Smegma[a]					
IA alone	64	68%	2,391	63%	0.1733
Smegma alone	1	1%	57	1%	0.3162
Smegma and IA	14	15%	294	8%	0.0006
Neither smegma or IA	15	16%	1,062	28%	ref
Women and Men					
Male and Female ulcers					
Male ulcer alone	46	24%	1,689	15%	0.0035
Female ulcer alone	9	5%	196	2%	0.0012
Male and Female Ulcers	7	4%	55	1%	<.0001
Neither male of female ulcer	130	68%	8,974	82%	ref
Female ulcer and Smegma[a]					
Smegma alone	11	12%	334	9%	0.0088
Female ulcer alone	6	6%	147	4%	0.0069
Smegma and female Ulcer	4	4%	17	0.40%	<.0001
Neither smegma or female ulcer	73	78%	3,306	87%	ref

[a] collected until 2002
*from unadjusted hazards ratios

Table 3. Adjusted hazard ratios for concurrent genital abnormalities and assessing for multiplicative interaction among M+F- couples in Lusaka, Zambia

	aHR[a]	95%CI[a]		p-value[a]	Expected Combined Effect (aHR)	Multiplicative interaction?[b]
Women						
BV and Genital discharge					**1.91**	**Yes, synergistic**
BV alone	1.03	0.52	2.02	0.9361		
Genital discharge alone	1.86	1.14	3.04	0.0129		
BV and genital discharge	2.59	1.08	6.19	0.0325		
Neither BV or genital discharge	ref					
Candida and Genital discharge					**2.71**	**Yes, synergistic**
Candida alone	1.52	0.85	2.71	0.1610		
Genital discharge alone	1.78	1.05	3.03	0.03217		
Candida and genital discharge	3.21	1.60	6.45	0.0010		
Neither Candida or genital discharge	ref					
Trichomoniasis and Genital discharge					**2.92**	**Yes, antagonistic**
Trichomoniasis alone	1.48	0.71	3.08	0.2980		
Genital discharge alone	1.97	1.24	3.15	0.0045		
Trichomoniasis and genital discharge	2.34	0.82	6.67	0.1115		
Neither Trichomoniasis or genital discharge	ref					
Candida and Trichomoniasis						
Candida alone	1.78	1.10	2.88	0.0193	**2.85**	**Yes, antagonistic**
Trichomoniasis alone	1.60	0.84	3.04	0.1526		
Candida and Trichomoniasis	2.15	0.29	15.73	0.4512		
Neither Candida or Trichomoniasis	ref					
Erosion/friability of the cervix/vagina and IA*					**7.12**	**Yes, antagonistic**
Erosion Alone	2.91	1.45	5.86	0.0028		
IA alone	2.45	1.50	3.98	0.0003		
Erosion and IA	2.01	0.48	8.44	0.3382		
Neither Erosion or IA	ref					
IA and Ulcer*					**4.91**	**Yes, antagonistic**
Ulcer Alone	2.06	1.10	3.87	0.0240		
IA alone	2.38	1.46	3.89	0.0005		
Ulcer and IA	2.07	0.50	8.55	0.3148		
Neither Ulcer or IA	ref					
BV and IA*					**2.68**	**Yes, antagonistic**
BV Alone	1.28	0.70	2.35	0.4189		
IA alone	2.09	1.26	3.47	0.0044		
BV and IA	1.93	0.60	6.25	0.2723		
Neither BV or IA	ref					

Genital discharge and IA*					**3.45**	**Yes, antagonistic**
Genital discharge alone	1.56	0.96	2.54	0.0742		
IA alone	2.21	1.27	3.84	0.0048		
Genital discharge and IA	2.21	0.97	5.06	0.0608		
Neither genital discharge or IA	ref					
Men						
Ulcer and Circumcision					**1.41**	**Yes, antagonistic**
Circumcision alone	0.89	0.46	1.72	0.7358		
Ulcer alone	1.58	1.10	2.25	0.0127		
Circumcision and Ulcer	1.10	0.34	3.58	0.8729		
Neither circumcision or ulcer	ref					
Genital discharge and Ulcer					**9.94**	**Yes, antagonistic**
Genital discharge alone	5.69	2.88	11.24	<.0001		
Ulcer alone	1.75	1.22	2.50	0.0023		
Genital discharge and ulcer	3.24	1.16	9.01	0.0243		
Neither genital discharge or ulcer	ref					
Genital discharge and IA*					**3.47**	**Yes, synergistic**
Genital discharge alone	2.48	0.93	6.59	0.0695		
IA alone	1.40	0.92	2.14	0.1146		
Genital discharge and IA	4.98	2.23	11.08	<.0001		
Neither genital discharge or IA	ref					
IA and Smegma*					**2.94**	**Yes, antagonistic**
IA alone	1.35	0.88	2.05	0.1690		
Smegma alone	2.18	0.84	5.70	0.1101		
Smegma and IA	2.16	1.17	4.01	0.0144		
Neither smegma or IA	ref					
Women and Men						
Male and Female Ulcers					**2.91**	**Yes, synergistic**
Male ulcer alone	1.47	1.02	2.11	0.0402		
Female ulcer alone	1.98	0.88	4.45	0.0971		
Male and Female Ulcers	4.78	2.00	11.44	0.0004		
Neither male of female ulcer	ref					
Female ulcer and Smegma*					**2.74**	**Yes, synergistic**
Smegma alone	1.49	0.88	2.53	0.1354		
Female ulcer alone	1.84	0.90	3.76	0.0958		
Smegma and female ulcer	5.34	1.87	15.23	0.0017		
Neither smegma or female ulcer	ref					

*collected until 2002

[a]adjusted for age, viral load of positive partner, time since enrollment in counseling, and inflammation if ulcerative exposure, ulceration if inflammatory exposure.

[b] If observed aHR differed from expected aHR by 10% or greater, multiplicative interaction occurred. Synergistic if observed aHR was greater than expected, antagonistic if observed aHR was less than expected.

Table 4. Population attributable fractions for genital abnormality exposures and pairs of genital abnormality exposures among M+F- couples in Lusaka, Zambia

	aHR	% of cases exposed	PAF
Women			
Genital Discharge	1.97	16%	8%
Bacterial Vaginosis (BV)	1.17	9%	1%
Candida	1.72	14%	6%
Trichomoniasis	1.50	8%	3%
Ulcer	2.47	8%	5%
Incident Syphilis (RPR)	1.69	4%	2%
Erosion/friability of cervix/vagina* (Erosion)	2.45	11%	6%
Inguinal adenopathy (IA)*	2.13	27%	14%
BV and genital discharge	2.59	4%	2%
Candida and genital discharge	3.21	6%	4%
Trichomoniasis and genital discharge	2.34	2%	1%
Candida and Trichomoniasis	2.15	1%	1%
Erosion and IA*	2.01	2%	1%
Ulcer and IA*	2.07	2%	1%
BV and IA*	1.93	5%	3%
Genital discharge and IA*	2.21	10%	5%
Men			
Genital Discharge	4.02	8%	6%
Ulcer	1.55	28%	10%
Inguinal adenopathy (IA)	2.02	83%	42%
Smegma	1.81	16%	7%
Circumcision and ulcer	1.10	2%	0%
Genital discharge and ulcer	3.24	2%	1%
Genital discharge and IA*	4.98	7%	6%
Smegma and IA*	2.16	15%	8%
Women + Men			
Male and Female ulcers	4.78	4%	3%
Smegma and Female ulcer*	5.34	4%	3%

*Collected before 2002

Chapter III. SUMMARY, PUBLIC HEALTH IMPLICATIONS, POSSIBLE FUTURE DIRECTIONS

HIV remains a pressing concern in sub-Saharan Africa. Addressing this disparity is integral for moving us towards meeting the United Nation's Sustainable Development Goal number 3: ensure healthy lives and promote well-being for all at all ages, and more specifically, to end the AIDS epidemic by 2030(33). Current methods in place to try and reduce the HIV disease burden regionally include provision of antiretroviral therapy (ART) and implementing services such as couples' voluntary counseling and testing (CVCT) where couples are educated about the importance of condom use and routine HIV testing. However, because there is not uniform accessibility to either ART or CVCT(7-9), other interventions are required to help reduce incident HIV infections.

Several STIs have been documented as co-factors in sexual HIV transmission and acquisition; identifying other co-factors in sexual HIV transmission could facilitate interventions that would reduce the HIV infection rate, particularly among stable heterosexual couples. Our study found that there are several non-sexually transmitted genital abnormalities that are associated with HIV acquisition among women in M+F-sero-discordant couples in Lusaka, Zambia. These genital abnormalities include: genital discharge, Candida infection, genital ulcer, erosion/friability of the cervix or vagina, and inguinal adenopathy in women, as well as genital discharge, genital ulcer, inguinal adenopathy, and smegma in men. We also observed synergistic GUI-GUI interactions for: BV in women with female genital discharge, Candida in women with female genital discharge, male discharge with male inguinal adenopathy (IA), concurrent male and

female genital ulcers, and female ulcer with male smegma, on their effect on risk of HIV acquisition.

Our study findings support the need for future research investigating the effect of non-sexually transmitted genital abnormalities in both partners as well as interaction between multiple STIs and non-STI genital abnormalities on risk of HIV acquisition. Future interventions focusing on increased routine screening and treatment of non-STI genital abnormalities among sero-discordant, heterosexual couples, and providing education for couples about identifying potential symptoms of genital abnormalities, particularly genital ulcers and genital discharge, and encouraging them to seek care to prevent increased risk of HIV acquisition, may be beneficial.