

Measuring HIV in the Female Genital Tract and How This Affects Sexual and Reproductive Choices

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During a routine office visit, a 30 year-old Liberian immigrant asks her doctor about her options for child bearing. She was diagnosed with HIV-1 infection when she came to the United States 5 years ago, and since then, she has begun taking antiretroviral therapy to control her infection. Her levels of HIV-1 as measured in blood plasma have been suppressed to undetectable levels, <50 copies, for 2 years and her immune system has been restored to normal levels of functioning with a CD 4 cell count of 600/mm³.

Since arriving in the United States, she has gotten married, and she and her new husband would like to start a family. When she was first diagnosed with HIV she was told she must always use condoms during intercourse to keep from passing her infection on to her sex partner who is HIV negative. Still, she knows other women with HIV who have had healthy children, and she has heard from others that when her virus is no longer detectable in her blood,

she can not transmit her infection to her husband or to her child.

Background

Impact of Gender on HIV Worldwide

In modern clinical practice, these are issues that physicians and patients must face on an increasingly regular basis. According to UNAIDS, there are more than 40 million people living with HIV today. Of these, 50% are women. In Africa, 57% of those with HIV-1 infection are women, and of those aged 15-24, more than 75% are women. According to the Centers for Disease Control and Prevention, of the more than 400,000 people living with AIDS in the US, 22% are women, and AIDS is among the leading causes of death among young African-American

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women. In 2002, 28% of US HIV cases diagnosed were women, but of those aged 13-24 years, 41% were women. The HIV epidemic is increasingly affecting women in their child-bearing years.

These are disturbing but predictable trends. In many places throughout the world, women find themselves in situations where they are unable to limit their exposure to HIV. In areas of conflict, rape is used as a method of intimidation and control. Under conditions of poverty and deprivation, women may find themselves in economic dependency that does not allow them any alternative to an abusive or dangerous relationship without risking starvation and homelessness for themselves and their children. Economic hardship may also force women to work in the commercial sex trade to support their families. In any of these situations, to suggest that women can require or even request the use of condoms or any other barrier method of protection is naïve at best.

Women trapped in these circumstances may at least be aware of their risks and could conceivably avail themselves of HIV testing and early diagnosis, with the hope of earlier treatment. What seems to be a much more common pattern however is that many women are at high risk for HIV infection without knowing it. We found in a chart review of 134 HIV-infected women receiving care at an HIV treatment center in India, that 88% were married to their only lifetime sexual partner.¹ We found a similar pattern in Cambodia, where HIV-infected women delivering their babies in a maternity hospital were statistically more likely to be married than women without HIV.² In these circumstances, it may not be possible for a woman to know her risk of

infection, and may not even consider HIV testing until she has advanced, symptomatic HIV or AIDS. The result is a large and growing pool of women with HIV, who are in their child-bearing years and who remain completely unaware of their infection. In other words, women are at high risk for becoming infected, often without classic risk factors such as multiple sexual partners or intravenous drug use. Once infected, if a woman is unaware of her infection, she may unwittingly transmit her infection to subsequent sex partners or to her children.

Sex as a Risk Factor for HIV Infection

It is generally accepted that the principal mode of HIV transmission between adults worldwide is through unprotected heterosexual contact. This is supported by a strong correlation between HIV infection and transmission and other sexually transmitted diseases (STDs).³ STDs have consistently been shown to increase the risk of transmitting HIV, as well as the risk of becoming infected with HIV. In addition, the treatment of STDs may actually reduce the rate of HIV transmission within a community when treatment programs are implemented early enough in an epidemic.⁴ Another indicator of the role sexual intercourse plays in the establishment of HIV epidemics is the finding that condom campaigns and reductions in other sexual risk behaviors can be effective in reducing the spread of HIV.⁵⁻⁹ In the United States, the proportion of AIDS cases attributable to heterosexual exposure has increased from 3% in 1985 to 31% in

2003.¹⁰ Among US women, sexual contact is responsible for 71% of all AIDS cases.

Based on studies from the United States and Europe, risk for sexual transmission was thought to be greater from male to female than from female to male.^{11,12} More recent data however suggest that the rates of female to male transmission is at least equal, if not higher than that of male to female transmission.^{13,14} A greater understanding of the dynamics of HIV in the female genital tract can help to explain why these differences exist, and how we can develop programs to reduce sexual transmission of HIV.

Mother to Child Transmission as a Risk Factor

For children, the greatest risk for HIV infection is being born to a woman with HIV. In the United States, 91% of children under the age of 13 acquired HIV around the time of birth.¹⁵ For non-breast fed infants, these infections most often occur late in pregnancy and as the infant passes through the birth canal, with exposure to blood and vaginal secretions containing HIV. This is confirmed by the numerous studies that show a marked decrease in mother-to-child transmission following cesarean delivery, which limits the exposure to genital tract HIV.¹⁶⁻²¹ One study from Kenya and another in Botswana found that the detection of HIV in the vagina of a pregnant woman was significantly associated with transmission of HIV to the infant.^{22,23}

Blood Levels of HIV and HIV Transmission

Among the many factors associated with

HIV transmission between sex partners and from mother to child, the one factor that stands out as being the strongest predictor is the level of HIV in the blood, or the plasma viral load. The best evidence for this came from a study of couples in Uganda.²⁴ More than 15,000 individuals participated in a study that was part of a larger trial for the prevention of STDs, for a period of 30 months. During the course of the study there were 90 new HIV infections that occurred between 415 couples. There was a step-wise increase in the rate of HIV transmission with every increase in plasma viral load in the transmitting partner.

A similar effect has been seen in mother to child transmission. In many studies, maternal plasma viral load is directly related to risk for HIV transmission to infant.²⁵⁻²⁷ With the use of effective drugs to control HIV, the risk of mother to child transmission can be reduced to nearly 1-2%. However, even with undetectable levels of HIV in the blood, transmissions still do occur. One possible explanation for these breakthrough transmissions is a discordance between blood and genital tract HIV levels. It may be possible in some circumstances to have significant amounts of HIV in the genital tract, despite undetectable blood levels.

Measuring HIV in the Female Genital Tract

Over the past 10 years, techniques have been developed to measure the amount of HIV present in cervical and vaginal areas. Several methods have been used to detect what most refer to as HIV genital shed-

ding. Specimens can be collected using a cervical and vaginal washing technique called a lavage, by absorbing secretions on filter paper, or by using a cotton-tipped swab. These specimens can then be used to determine if HIV is present and in what quantities. Using these techniques, it is even possible to sample individual parts of the genital tract to see which part of the genital tract contains more virus. Once HIV is found, it is also possible to determine if these viruses contain mutations which make them less susceptible to medications used for treatment. Identifying drug-resistant HIV in the genital tract suggests these viruses may be ready to be sexually transmitted.^{28,29}

The Determinants of Genital Tract HIV Shedding

Several factors have been shown to contribute to genital tract HIV shedding. As it turns out, the same predictor for sexual and mother to child transmission, plasma viral load, is also the most reliable predictor for genital tract shedding.^{30,31} Using multiple sampling techniques, Kovacs and colleagues found that more than 3/4 of women with detectable plasma HIV also had detectable genital tract HIV. A high rate of genital tract HIV shedding among women not receiving effective antiretroviral therapy has been demonstrated by several other groups in a variety of settings. Our group found HIV shedding among 60% of antiretroviral naïve women in the Philippines and more recently, McLean and colleagues found that 81% of untreated women in Northern Thailand had detectable HIV in the genital tract.^{32,33} Shedding is therefore a common event among women with de-

tectable blood plasma HIV.

Genital Infections and HIV Shedding

Multiple studies have demonstrated that concomitant infections in the genital tract of both men and women increase the frequency and degree of HIV shedding.³⁴⁻³⁷ Of particular interest are herpes simplex virus (HSV) infections and bacterial vaginosis (BV). Herpes simplex is important in its contribution to HIV shedding because it is extremely common among women with HIV. It can be treated but can not be cured, so frequent recurrences are possible. Bacterial vaginosis is also common: at any one time, up to 1/3 of all women will have BV. BV can be cured but frequently recurs, even in the absence of sexual contact.

Herpes simplex has a long epidemiologic association with HIV infection.³⁸ The population prevalence of HSV type 2, the primary genital type, varies but is roughly 20% in the United States.³⁹ Among high-risk populations, the prevalence of HSV-2 is much higher. Among HIV infected women in the United States, the prevalence of HSV-2 by serologic testing for antibody is 78%, and among female commercial sex workers in Senegal, the prevalence was found to be 96%.^{40,41} Not only is HSV-2 common among women at risk for or already living with HIV, HSV-2 has been shown to facilitate HIV shedding among women with both infections.^{42,43} This association has been seen both with symptomatic herpes outbreaks and even with asymptomatic herpes. Herpes simplex therefore appears to increase the infectiousness of HIV, even among women without symptoms of herpes infection.

Medications such as acyclovir have been shown to reduce HSV shedding by 90%.⁴⁴ Studies are ongoing to determine if use of these medications can prevent HIV genital tract shedding and subsequent transmission.

Like HSV, BV is an extremely common disorder, particularly among populations of women where HIV is prevalent. In one cross-sectional study in rural Uganda, 6456 women were tested for the presence of genital infections.⁴⁵ Fifty one percent had BV, and 80% of them were without symptoms. BV has also been shown to increase genital tract HIV shedding.^{37,46} In one study in Italy, women were more than 3 times more likely to have HIV shedding if they had BV. In the United States, our group has found that HIV-infected women with BV were almost 6 times more likely to shed HIV.

In addition to BV and HSV, other genital infections have also been associated with HIV shedding. In a study in Cote d'Ivoire, HIV shedding was more common among women with gonorrhea, chlamydia and genital ulcer.³⁵ The prevalence of shedding declined from 42% to 21% among women whose genital infection was cured with treatment.

Attempts at controlling genital infections with antibiotic treatments in order to reduce HIV infections have met with varying success.^{4,47,48} A reasonable explanation for the lack of efficacy of some programs is the failure to treat the most common genital infections effectively. Given the relative frequency of HSV and BV infections and the need for continuous or frequent treatments to control them, the effect of other interventions may be overwhelmed by the degree and frequency of HIV shedding as-

sociated with these 2 conditions.

Treatment for HIV and Shedding

Antiretrovirals, used for the treatment of HIV, are extremely potent at reducing HIV levels in the blood, often to undetectable levels. With these decreases in blood plasma HIV, similar decreases in genital tract HIV levels are seen. Our group has shown that within days of starting HIV treatment, levels of HIV in the genital tract begin to decline.⁴⁹ In a study of 7 women beginning therapy who had daily genital tract samples collected, all subjects had undetectable levels of HIV in the genital tract by the 14th day. With sustained suppression of plasma viral load, genital tract viral loads also tend to remain suppressed, but not always. In studying a series of 20 women with sustained suppression of plasma HIV, we found that 8 women had intermittent periods of shedding in the genital tract.⁵⁰ The suppression of genital tract HIV using antiretroviral therapy is effective, but unpredictable breakthrough shedding often occurs.

Complete, durable suppression of HIV in the blood is by no means assured with the use of antiretroviral drugs. In general practice, up to 1/3 of all patients on treatment will experience breakthrough increases in plasma viral load. When this happens, there are often drug-resistant mutant viruses present. Our group found in one study of genital tract virus among women who were taking non-suppressive antiretroviral therapy, 4 out of 7 women had mutant virus present in the genital tract that differed from the mutants found in blood.⁵¹ One possible explanation for

the differences in the expression of drug-resistant mutants between the genital tract and the blood is that antiretroviral drug concentrations differ in different body compartments. Sub-therapeutic drug concentrations in the genital compartment may create an environment where HIV is allowed to replicate and evolve drug resistance in a site where these resistance mutations may be readily transmitted. Our group and colleagues from the University of North Carolina have found that concentrations of 8 different antiretroviral drugs in the female genital tract varied from <10% to >100% of those found in the blood plasma.⁵²

Other Factors Associated with HIV Shedding

Several other factors or conditions have been associated with HIV shedding in the female genital tract. In Mombasa, Kenya, 213 women were examined for an average of 64 days after starting hormonal contraception.⁵³ A modest but significant increase in genital tract HIV shedding was noted, although this did not correlate to any particular type of contraception. These findings are similar to a cross-sectional study in Mombasa, which found that women receiving hormonal contraception had a greater likelihood of genital tract shedding compared to women not receiving these treatments.⁵⁴ HIV shedding has been seen to fluctuate according to the menstrual cycle as well. An increase in HIV shedding occurs just before the start of menses.⁵⁵ This may be due to changes in vaginal cytokine levels, which appear to increase significantly during menses but are not associated with any changes in the level of HIV

in the blood.⁵⁶ Since cytokines are responsible for increasing inflammation and can stimulate viral production, this observation is not unexpected. Another cross-sectional study in Italy found a similar relationship between vaginal cytokines and HIV shedding, and found that antiretroviral therapy reduced the inflammatory cytokine levels.⁵⁷ The induction of inflammation by the daily use of the topical product nonoxynol-9 has been implicated in the increase of genital lesions and a trend toward an increase in genital tract HIV shedding among women using this product in randomized trials.⁵⁸ More detailed analysis of cytokines and examinations for clinical evidence of inflammation or toxicity are an important part of all studies evaluating topical vaginal products.

One confusing area that is still under investigation is the association between micronutrient deficiency and HIV shedding. Several studies have found an association between micronutrient deficiency and HIV shedding and others have shown an increase in mother to child HIV transmission rates among women deficient in vitamin A.^{54,59-61} However, supplementation has at best demonstrated little effect on genital tract HIV shedding or mother to child transmission, and in some studies actually increased the risk of transmission through breastfeeding and increased genital tract HIV shedding.⁶²⁻⁶⁵

Future Directions

We have seen that there are increasing numbers of women of reproductive age who are becoming infected with HIV. We have also seen that many of these women

are unaware of their infection, or even their risk for having HIV. Finally we have seen that there are many factors that contribute to shedding of HIV in the female genital tract which can increase a woman's risk for transmitting her infection to her sex partners or children. Many of these factors are modifiable such that, with proper intervention, genital tract HIV shedding can be reduced and transmission prevented. Testing these interventions is an important area for future prevention research.

One area of particular attention is the regular screening and treatment of genital tract infections. While it is widely recognized that treatment of genital infections can reduce HIV transmissions, the optimum implementation of programs to realize the maximum benefit remains elusive. Programs that accurately diagnose genital infections in women and provide effective treatments for the most prevalent genital infections have not yet been sufficiently tested for their impact, let alone implemented on a sufficient scale.

In addition to treatment of genital infections, the use of antiretroviral medications for the prevention of HIV transmission has yet to be adequately studied. While it is clear that these medications do lower viral loads and infectiousness, the prevention effect could be diluted by an increase in sexual risk taking by individuals believing they are less infectious and by the increased number of individuals now able to survive with HIV infections as a consequence of effective treatments. While those individuals with severely weakened immune systems are clearly in need of medications, research is currently being conducted to determine whether treatment of asymptomatic individuals at


earlier disease stages is an effective strategy to prevent transmissions.

One of the most effective prevention interventions, the latex condom, is not available to many vulnerable women. New methods of female-controlled protection, such as topical microbicides will be a major advance for HIV prevention programs. Despite the failure of nonoxynol-9 as one such product, a number of others are advancing through the clinical trials pipeline. An effective product might not only prevent a woman from being infected, but might also prevent an infected women from transmitting HIV to her sex partners. Measurement of HIV shedding in women using these products is an important component of the development process for these products.

Advice to an HIV Infected Woman Wishing to Become Pregnant

We began our discussion with a vignette that we are encountering with increasing frequency. With undetectable levels of HIV in the blood, the risks for transmission from our patient to her sex partner and baby through genital tract shedding is quite low. In the Ugandan couples study, there were no transmissions observed between sex partners when blood HIV levels were below 1500 copies per milliliter.²⁴ However, we have shown that genital tract shedding does occur among women even with undetectable levels of HIV in the blood, and this shedding is unpredictable. Transmission of HIV from mother to child can also occasionally occur even when plasma viral load is undetectable. For our pa-

tient wishing to become pregnant with an uninfected partner, she should be advised to consider artificial insemination to avoid exposing her partner to even this low risk. With undetectable plasma HIV levels she has a 1-2% chance of transmitting HIV to her infant. Women with detectable levels of HIV in the blood despite antiretroviral therapy may choose to undergo elective cesarean delivery to minimize the risk of exposure through genital tract HIV shedding.

To summarize, the shedding of HIV from the female genital tract is common among women not receiving antiretroviral therapy. When this shedding does occur, it is intermittent and unpredictable. The best predictor of genital tract HIV shedding is blood plasma viral load. There are ways to intervene to reduce genital tract HIV shedding, through the treatment of genital infections, the use of antiretroviral drugs, and possibly through the use of microbicides or other therapies. There are clear instances of discordance between plasma and genital tract HIV levels, and we need to study these further to understand how to predict this phenomenon, what other factors affect shedding, and how to prevent it. 

References

1. Newmann S, Sarin P, Kumarasamy N, et al. Marriage, monogamy and HIV: a profile of HIV-infected women in south India. *Int J STD AIDS* 2000;11(4):250-3.
2. Theng T, Sok P, Pugatch D, et al. HIV-1 cord-blood seroprevalence of parturient women in Sihanoukville, Cambodia. *Int J STD AIDS* 2004;15(6):419-21.
3. Fleming DT, Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sex Transm Infect* 1999;75(1):3-17.
4. Grosskurth H, Mosha F, Todd J, et al. Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. *Lancet* 1995;346(8974):530-6.
5. Asiimwe-Okiror G, Opio AA, Musinguzi J, Madraa E, Tembo G, Carael M. Change in sexual behaviour and decline in HIV infection among young pregnant women in urban Uganda. *Aids* 1997;11(14):1757-63.
6. Mason CJ, Markowitz LE, Kitsiripornchai S, et al. Declining prevalence of HIV-1 infection in young Thai men. *Aids* 1995;9(9):1061-5.
7. Hanenberg RS, Rojanapithayakorn W, Kunasol P, Sokal DC. Impact of Thailand's HIV-control programme as indicated by the decline of sexually transmitted diseases. *Lancet* 1994;344(8917):243-5.
8. Ahmed S, Lutalo T, Wawer M, et al. HIV incidence and sexually transmitted disease prevalence associated with condom use: a population study in Rakai, Uganda. *Aids* 2001;15(16):2171-9.
9. Cohen J. Asia--the next frontier for HIV/AIDS. Two hard-hit countries offer rare success stories: Thailand and Cambodia. *Science* 2003;301(5640):1658-62.
10. Anonymous. AIDS General Epidemiology Slide Set Through 2003: Centers for Disease Control and Prevention, 2005.
11. Nicolosi A, Correa Leite ML, Musicco M, Arici C, Gavazzeni G, Lazzarin A. The efficiency of male-to-female and female-to-male sexual transmission of the human immunodeficiency virus: a study of 730 stable couples. Italian Study Group on HIV Heterosexual Transmission. *Epidemiology* 1994;5(6):570-5.
12. Padian NS, Shiboski SC, Jewell NP. Female-to-male transmission of human immunodeficiency virus. *Jama* 1991;266(12):1664-7.
13. O'Farrell N. Enhanced efficiency of female-to-male HIV transmission in core groups in developing countries: the need to target men. *Sex Transm Dis* 2001;28(2):84-91.
14. Gray RH, Wawer MJ, Brookmeyer R, et al. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *Lancet* 2001;357(9263):1149-53.
15. Anonymous. Pediatric HIV/AIDS Surveillance Slide Set Through 2003: Centers for Disease Control and Prevention, 2004.
16. Caesarean section and risk of vertical transmission of HIV-1 infection. The European Collaborative Study. *Lancet* 1994;343(8911):1464-7.
17. Bobat R, Coovadia H, Coutsooudis A, Moodley D. Determinants of mother-to-child transmission of human immunodeficiency virus type 1 infection in a cohort from Durban, South Africa. *Pediatr Infect Dis J* 1996;15(7):604-10.
18. Kuhn L, Bobat R, Coutsooudis A, et al. Cesarean deliveries and maternal-infant HIV transmission: results from a prospective study in South Africa. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;11(5):478-83.
19. Maguire A, Sanchez E, Fortuny C, Casabona J. Potential risk factors for vertical HIV-1 transmission in Cat-

- alonia, Spain: the protective role of cesarean section. The Working Group on HIV-1 Vertical Transmission in Catalonia. *Aids* 1997;11(15):1851-7.
20. Kind C, Rudin C, Siegrist CA, et al. Prevention of vertical HIV transmission: additive protective effect of elective Cesarean section and zidovudine prophylaxis. Swiss Neonatal HIV Study Group. *Aids* 1998;12(2):205-10.
 21. Mandelbrot L, Le Chenadec J, Berrebi A, et al. Perinatal HIV-1 transmission: interaction between zidovudine prophylaxis and mode of delivery in the French Perinatal Cohort. *Jama* 1998;280(1):55-60.
 22. John GC, Nduati RW, Mbori-Ngacha DA, et al. Correlates of mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission: association with maternal plasma HIV-1 RNA load, genital HIV-1 DNA shedding, and breast infections. *J Infect Dis* 2001;183(2):206-212.
 23. Montano M, Russell M, Gilbert P, et al. Comparative prediction of perinatal human immunodeficiency virus type 1 transmission, using multiple virus load markers. *J Infect Dis* 2003;188(3):406-13.
 24. Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. *N Engl J Med* 2000;342(13):921-9.
 25. Sperling RS, Shapiro DE, Coombs RW, et al. Maternal viral load, zidovudine treatment, and the risk of transmission of human immunodeficiency virus type 1 from mother to infant. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. *N Engl J Med* 1996;335(22):1621-9.
 26. Mofenson LM, Lambert JS, Stiehm ER, et al. Risk factors for perinatal transmission of human immunodeficiency virus type 1 in women treated with zidovudine. Pediatric AIDS Clinical Trials Group Study 185 Team. *N Engl J Med* 1999;341(6):385-93.
 27. Mayaux MJ, Dussaix E, Isopet J, et al. Maternal virus load during pregnancy and mother-to-child transmission of human immunodeficiency virus type 1: the French perinatal cohort studies. SEROGEST Cohort Group. *J Infect Dis* 1997;175(1):172-5.
 28. Hecht FM, Grant RM, Petropoulos CJ, et al. Sexual transmission of an HIV-1 variant resistant to multiple reverse-transcriptase and protease inhibitors. *N Engl J Med* 1998;339(5):307-11.
 29. Yerly S, Kaiser L, Race E, Bru JP, Clavel F, Perrin L. Transmission of antiretroviral-drug-resistant HIV-1 variants. *Lancet* 1999;354(9180):729-33.
 30. Uvin SC, Caliendo AM. Cervicovaginal human immunodeficiency virus secretion and plasma viral load in human immunodeficiency virus-seropositive women. *Obstet Gynecol* 1997;90(5):739-43.
 31. Kovacs A, Wasserman SS, Burns D, et al. Determinants of HIV-1 shedding in the genital tract of women. *Lancet* 2001;358(9293):1593-601.
 32. Natividad-Villanueva GU, Santiago E, Manalastas RM, Jr., et al. Human immunodeficiency virus in plasma and cervicovaginal secretions in Filipino women. *Int J STD AIDS* 2003;14(12):826-9.
 33. McLean C, Chaowanachan T, Evans-Strickfaden T, et al. A high detection rate of cell-free cervicovaginal HIV among HIV-infected women enrolled in a vaginal microbicide trial in Northern Thailand. 12th Conference on Retroviruses and Opportunistic Infections. February 22-25 2005, Boston, MA: Abstract 537.
 34. Kreiss J, Willerford DM, Hensel M, et al. Association between cervical inflammation and cervical shedding of human immunodeficiency virus DNA. *J Infect Dis* 1994;170(6):1597-601.
 35. Ghys PD, Fransen K, Diallo MO, et al. The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Cote d'Ivoire. *Aids* 1997;11(12):F85-93.
 36. Wang CC, McClelland RS, Reilly M, et al. The effect of treatment of vaginal infections on shedding of human immunodeficiency virus type 1. *J Infect Dis* 2001;183(7):1017-22.
 37. Cu-Uvin S, Hogan JW, Caliendo AM, Harwell J, Mayer KH, Carpenter CC. Association between bacterial vaginosis and expression of human immunodeficiency virus type 1 RNA in the female genital tract. *Clin Infect Dis* 2001;33(6):894-6.
 38. Stamm WE, Handsfield HH, Rompalo AM, Ashley RL, Roberts PL, Corey L. The association between genital ulcer disease and acquisition of HIV infection in homosexual men. *Jama* 1988;260(10):1429-33.
 39. Fleming DT, McQuillan GM, Johnson RE, et al. Herpes simplex virus type 2 in the United States, 1976 to 1994. *N Engl J Med* 1997;337(16):1105-11.
 40. Hook EW, 3rd, Cannon RO, Nahmias AJ, et al. Herpes simplex virus infection as a risk factor for human immunodeficiency virus infection in heterosexuals. *J Infect Dis* 1992;165(2):251-5.
 41. Nahmias AJ, Lee FK, Beckman-Nahmias S. Sero-epidemiological and -sociological patterns of herpes simplex virus infection in the world. *Scand J Infect Dis Suppl* 1990;69:19-36.
 42. Baeten JM, McClelland RS, Corey L, et al. Vitamin A supplementation and genital shedding of herpes simplex virus among HIV-1-infected women: a randomized clinical trial. *J Infect Dis* 2004;189(8):1466-71.
 43. McClelland RS, Wang CC, Overbaugh J, et al. Association between cervical shedding of herpes simplex virus and HIV-1. *Aids* 2002;16(18):2425-30.
 44. Wald A, Zeh J, Barnum G, Davis LG, Corey L. Suppression of subclinical shedding of herpes simplex virus type 2 with acyclovir. *Ann Intern Med* 1996;124(1 Pt 1):8-15.
 45. Paxton LA, Sewankambo N, Gray R, et al. Asymptomatic non-ulcerative genital tract infections in a rural Ugandan population. *Sex Transm Infect* 1998;74(6):421-5.
 46. Spinillo A, Debiaggi M, Zara F, Maserati R, Polatti F, De Santolo A. Factors associated with nucleic acids related to human immunodeficiency virus type 1 in cervico-vaginal secretions. *Bjog* 2001;108(6):634-41.

47. Kaul R, Kimani J, Nagelkerke NJ, et al. Monthly antibiotic chemoprophylaxis and incidence of sexually transmitted infections and HIV-1 infection in Kenyan sex workers: a randomized controlled trial. *Jama* 2004;291(21):2555-62.
48. Wawer MJ, Sewankambo NK, Serwadda D, et al. Control of sexually transmitted diseases for AIDS prevention in Uganda: a randomised community trial. Rakai Project Study Group. *Lancet* 1999;353(9152):525-35.
49. Cu-Uvin S, Caliendo AM, Reinert S, et al. Effect of highly active antiretroviral therapy on cervicovaginal HIV-1 RNA. *Aids* 2000;14(4):415-21.
50. Cu-Uvin S, B. S, Hogan JW, et al. Episodes of detectable intermittent HIV-1 RNA in the genital tract of women with sustained undetectable plasma viral load (<80 copies/mL). 42nd IDSA Annual Meeting September 30-October 4, 2004, Boston, MA: Abstract 940.
51. De Pasquale MP, Leigh Brown AJ, Uvin SC, et al. Differences in HIV-1 pol sequences from female genital tract and blood during antiretroviral therapy. *J Acquir Immune Defic Syndr* 2003;34(1):37-44.
52. Min SS, Corbett AH, Rezk N, et al. Protease Inhibitor and Nonnucleoside Reverse Transcriptase Inhibitor Concentrations in the Genital Tract of HIV-1-Infected Women. *J Acquir Immune Defic Syndr* 2004;37(5):1577-1580.
53. Wang CC, McClelland RS, Overbaugh J, et al. The effect of hormonal contraception on genital tract shedding of HIV-1. *Aids* 2004;18(2):205-9.
54. Mostad SB, Overbaugh J, DeVange DM, et al. Hormonal contraception, vitamin A deficiency, and other risk factors for shedding of HIV-1 infected cells from the cervix and vagina. *Lancet* 1997;350(9082):922-7.
55. Benki S, Mostad SB, Richardson BA, Mandaliya K, Kreiss JK, Overbaugh J. Cyclic shedding of HIV-1 RNA in cervical secretions during the menstrual cycle. *J Infect Dis* 2004;189(12):2192-201.
56. Al-Harthi L, Kovacs A, Coombs RW, et al. A menstrual cycle pattern for cytokine levels exists in HIV-positive women: implication for HIV vaginal and plasma shedding. *Aids* 2001;15(12):1535-43.
57. Zara F, Nappi RE, Brerra R, Migliavacca R, Maserati R, Spinillo A. Markers of local immunity in cervico-vaginal secretions of HIV infected women: implications for HIV shedding. *Sex Transm Infect* 2004;80(2):108-12.
58. Rustomjee R, Abdool Karim Q, Abdool Karim SS, Laga M, Stein Z. Phase 1 trial of nonoxynol-9 film among sex workers in South Africa. *Aids* 1999;13(12):1511-5.
59. Baeten JM, Mostad SB, Hughes MP, et al. Selenium deficiency is associated with shedding of HIV-1--infected cells in the female genital tract. *J Acquir Immune Defic Syndr* 2001;26(4):360-4.
60. Semba RD, Miotti PG, Chipangwi JD, et al. Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. *Lancet* 1994;343(8913):1593-7.
61. John GC, Nduati RW, Mbori-Ngacha D, et al. Genital shedding of human immunodeficiency virus type 1 DNA during pregnancy: association with immunosuppression, abnormal cervical or vaginal discharge, and severe vitamin A deficiency. *J Infect Dis* 1997;175(1):57-62.
62. Baeten JM, McClelland RS, Overbaugh J, et al. Vitamin A supplementation and human immunodeficiency virus type 1 shedding in women: results of a randomized clinical trial. *J Infect Dis* 2002;185(8):1187-91.
63. Fawzi WW, Msamanga G, Hunter D, et al. Randomized trial of vitamin supplements in relation to vertical transmission of HIV-1 in Tanzania. *J Acquir Immune Defic Syndr* 2000;23(3):246-54.
64. Fawzi WW, Msamanga GI, Hunter D, et al. Randomized trial of vitamin supplements in relation to transmission of HIV-1 through breastfeeding and early child mortality. *Aids* 2002;16(14):1935-44.
65. McClelland RS, Baeten JM, Overbaugh J, et al. Micronutrient Supplementation Increases Genital Tract Shedding of HIV-1 in Women: Results of a Randomized Trial. *J Acquir Immune Defic Syndr* 2004;37(5):1657-1663.