TREATMENT FAILURE OF *TRICHOMONAS VAGINALIS* AMONG HIV+ WOMEN: RISK FACTORS AND METHODS

A DISSERTATION

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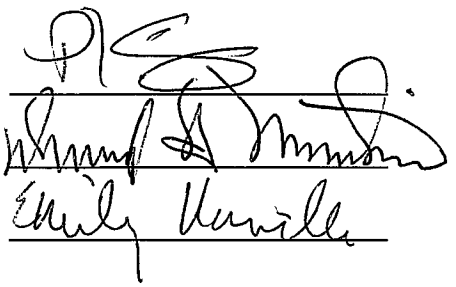

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Abstract

Background: Trichomoniasis is the most common non-viral sexually transmitted infection with worldwide; it is particularly prevalent amongst HIV positive women. The infection is commonly considered easily treatable, yet repeat infection rates in HIV positive women can be as high as 36%.

The overall objectives of this thesis is to examine the influence of select factors on treatment failure and retest positive of *Trichomonas vaginalis* (TV) among HIV+ women and to examine methodologies for eliciting reports of sexual behaviors.

Methods: A secondary data analysis was performed on a multi-centered cohort of HIV+/TV+ women who were randomized to single-dose (2g) or 7 day (500mg BID) multi-dose metronidazole treatment. Test of cure visit occurred 6-12 days after treatment completion and women returned for a 3-month re-test visit. To assess sexual behavior methods a literature search was conducted for articles that compared prospective and recall methods.

Results: Women on ART had higher retest positive than women not on ART with an increased relative risk of 2.63 (95% CI 1.04-6.63, p=0.036). Of the 143 women included for the assessment of smoking, the higher level of smoking had an increased risk of TV compared to non-smokers 3.44 RR (95% CI 1.31-9.04, p-value=0.012) and Cochran-Armitage trend test at p=0.007 between increasing levels of smoking and TV+.

Out of an initial 3140 articles, 14 met all the criteria for review. Among heterosexuals, recall of 1 month of sexual behavior was found to be similar to data collected more frequently (i.e. daily, over the same time span).

Conclusions: Among HIV positive women, ART usage and cigarette smoking may interfere with treatment of TV. Monthly recall methods appear to be similar to daily measures of sexual behavior among heterosexuals.

BACKGROUND AND SIGNIFICANCE

Trichomonas vaginalis

Trichomoniasis is the most common non-viral sexually transmitted infection with around 248 million estimated new cases worldwide in adults between the ages of 15 and 49 in 2005.[1] In 2008, the US estimated the prevalence to be 3,710,000 cases among men and women.[2] African-American women have prevalence rates of 13%,[3] which is disproportionate compared to the U.S. population-based prevalence of 3%.[4] In contrast to other STIs that have a higher prevalence in young adults, the rates of trichomoniasis are similar among all age groups of sexually active women. Even though TV is far more common than chlamydia and gonorrhea combined, and despite being rapidly diagnosed and treated, TV is not a reportable infection. TV has been evaluated as a potential reportable disease but only met 3 out of the 7 criteria for public health surveillance (frequency, communicability, and associated disparities).[5]

The causative agent of trichomoniasis is the anaerobic, flagellated protozoan *Trichomonas vaginalis* (TV). Extragenital infections (i.e. oral or anal) are rare, as TV preferentially infects squamous epithelium in the urogenital tract, vagina, urethra, and paraurethral glands. Less commonly, it has been found in the cervix, bladder, Bartholin glands, and prostate. The incubation time is usually 4 to 28 days after infection till the onset of symptoms.[6]

Epidemiology of TV and HIV co-infections

Among HIV + women, TV is the most common curable sexually transmitted infection (STI).[1] Once thought to be a nuisance infection, there is

mounting evidence that TV interacts with the acquisition and spread of HIV bringing TV to the forefront of STI research. HIV positive women can have prevalence rates ranging from 6%-53%.[3,7-13] TV has been associated with increased genital shedding of HIV, pelvic inflammatory disease, and adverse pregnancy outcomes.[3,4,10,14-17] Successful treatment of TV has been shown to reduce the genital shedding of HIV and thus may be an effective prevention strategy in reducing the incidence of HIV transmission.[11,18] High rates of repeat TV infections have been reported in HIV + women (18-36%) suggesting that HIV + women may have biological differences compared to HIV negative women that interfere with the standard of care treatment of single dose metronidazole (MTZ).[3,4,10,14-17] There is growing evidence that the standard of care single dose therapy is inadequate for preventing clinical treatment failure in HIV+ women.[19-21]

TV and HIV acquisition

Numerous studies have found a positive association between TV infection and HIV acquisition.[22-33] TV can increase susceptibility to HIV by causing disruptions in the cellular barrier and an increased inflammatory response.[34] An active TV infection may cause punctate mucosal hemorrhages which compromises the mechanical barrier to HIV entry. In the presence of TV, the vaginal innate immunity may become suppressed as TV has been seen to decrease innate immune factors.[35] The inflammation induced by the presence of TV increases the localization of HIV target cells, CD4 T lymphocytes.[36] The interaction of TV on a host's immunity increases the likelihood of a positive HIV infection when exposed. Quinlivan et al. estimated 23% of HIV transmission

events may be attributable to TV infection when 22% of women are also infected with TV.[37] This suggests that controlling the TV in a population may lower the number of new cases of HIV transmission, especially among African-American women.

TV and HIV shedding

Genital shedding of HIV may increase the likelihood of HIV transmission, though the precise amount of genital viral load necessary for transmission is currently unknown. Vaginal shedding is influenced by antiretroviral therapy, plasma viral load, douching, co-infection with STIs, bacterial vaginosis and unprotected sex with an HIV+ partner.[7] Even when plasma viral load is controlled by using ART, it is still possible for there to be vaginal shedding of HIV.[38] In women co-infected with TV and HIV, studies have shown that there is a decrease in the amount of vaginal shedding after TV was successfully treated. [38-42] The role of TV in increasing HIV genital shedding is as of yet unclear, but evidence is mounting of its importance.

Transmission

The primary mode of transmission is heterosexual intercourse. Women can also transmit the infection to their female sex partners, but it is unlikely that men infect their male sex partners.[43] The organism does not thrive outside the body and can only survive in a wet environment for up to three hours.[44-46] There is evidence of survival on fomites and there have been a few possible cases of fomites or water being the vehicle of infection.[47,48] Women are more highly affected than men. In women, the infection can persist for months or for several

years, especially if the infection is asymptomatic. However, without treatment 54%-69% of men may spontaneously clear infection.[39,49,50] The transient nature of the infection in men may be due to the cytotoxic effect of the zinc-rich prostatic fluid.[51] Rectal infections have not been detected.

Signs and Symptoms

While in it has been estimated that among women being screened at sexual health clinics, up to 50% with TV are asymptomatic[52], when the signs and symptoms of an infection are present they are often green-yellow frothy vaginal discharge, pain on sexual intercourse, vulvovaginal soreness and itching, and pain on urination. Though rarely seen in a visual examination, with colposcopy evaluation nearly 50% of women have TV-induced *Coplitis macularis* or strawberry cervix.[53] Clinical evaluation is not sufficient for diagnosis and laboratory confirmation is necessary to determine current infection. Untreated TV can persist for months or years. Trichomoniasis infections can be misdiagnosed as bacterial vaginosis (BV) if laboratory tests are not preformed, due to BV and TV presenting similarly when healthcare providers are limited to assessment using Amsel criteria (thin, white, yellow, homogeneous discharge, clue cells on microscopy, pH of vaginal fluid >4.5, and a fishy odor on addition of 10% potassium hydroxide solution).[54]

Complications

Trichomoniasis infections are associated with several complications. Women who are pregnant can have premature rupture of membranes[55], preterm delivery, low birth rate[56], and infant mortality. It has also been associated with

cervical cancer. In immunocompromised individuals who are exposed orally, TV can cause pneumonia, bronchitis, and oral lesions.[57] Women with TV who undergo gynecologic surgery can be at increased risk of infections from other organisms.[58,59] TV is also a risk factor for the development of post-hysterectomy cuff cellulitis.[60] In women co-infected with HIV there is an association between TV and pelvic inflammatory disease.[61] Active infections with TV can increase the risk of a woman being infected with HIV.[20,62] TV can cause punctate mucosal hemorrhages which results in the mechanical barrier to HIV to weaken.[63] Due to the inflammation of the vaginal tissue caused by TV, there is an increase in the local number of HIV target cells.[64] The two complications combined with a change in the normal vaginal flora, as is often seen in TV, increase the ability of the HIV virus to cause infection. Men infected with TV can have nongonococcal urethritis, prostatitis, and can have a decrease in sperm motility and viability.[65]

Diagnostic Testing

The goal of testing is to identify individuals who have been re-infected or have experienced a treatment failure. Identification of those individuals who remain TV positive after treatment is important in order to facilitate their re-treatment and treatment of their sexual partners. If TV can be successfully eliminated from a patient's sexual network, it is unlikely that they will be re-infected unless new partners from outside their network are added. With each method of testing for the presence of TV there are certain problems that arise.

There are several methods for testing genital secretions for the presence of TV. The traditional method, and most common worldwide, is the wet mount

smear, where a swab of vaginal fluid is mixed with saline solution and observed under light microscopy. The TV organism is slightly larger than a white blood cell and is oval with 5 flagella. The advantages for wet mount testing are that it is a cheap and quick diagnostic test that can be performed in the clinic setting and have results immediately available. The disadvantages are that the sensitivity and specificity of the test are highly dependent on the skill of the technician; sensitivity can be as low as 50%.[66] False positives can result from inexperienced readers mistaking Brownian motion (the random motion of particles suspended in a fluid) for the distinctive "corkscrew" motility of the TV organism. For correct diagnosis the TV must be mobile on the slide. Wet mount reading are also dependent on the amount and health of the parasites present in the vagina. If the parasitic load is too low or the TV are weakened from ineffective treatment, they may not be present in the wet mount or may not be moving enough for detection to occur. Due to the low sensitivity of wet mount, it is advisable to use another method of detection.

Culturing the TV parasite from vaginal swabs is considered the current gold standard for diagnosis. The specificity is high, near 100%, and the sensitivity is between 83-94%,[67] though there have been reports that the sensitivity can be as low as 68.8%[68] instead of the 83-94% previously reported. Swabs of genital secretions are inoculated into culture media and incubated for several days to facilitate the growth of the parasitic load. The culture media inhibits the growth of unwanted organisms, i.e. bacteria and yeasts that are common in vaginal flora. Culture methods are more time-consuming and expensive; incubated cultures

are read daily for up to 3 days to determine a negative result. The cost for the culture and technician time may be cost prohibitive for public clinics. While the culture has improved sensitivity compared to wet mount in women, it has demonstrated poor sensitivity in men. Due to the incubation time, because this is not a point of care test, there is the possibility that a patient whose culture is positive after a few days may be difficult to contact. Even if contact is made it may be difficult for them to return to the clinic for treatment and counseling.

Point of care tests have the advantage of having improved sensitivity, compared to wet mount, and the results are available in minutes. The two approved tests are OSOM Trichomonas Rapid Test (Genzyme Diagnostics; Cambridge, MA)[69] and Affirm VP III (Becton, Dickinson & Co.; Franklin Lakes, NJ.[70] The OSOM test is an immunochromatographic capillary flow dipstick technology in which the presence of TV is detected by conjugated antigen complexes. Results from OSOM are available within 10 minutes. Compared to culture, the OSOM test has a sensitivity of 83% and a specificity of 99%. OSOM tests for the presence of a TV antigen and not for the live organism. A patient who has been treated for TV in the past few weeks may show a false positive on the OSOM test as the antigen may still be present in the vagina. This test is only approved for testing in women.

The Affirm test is a nucleic acid probe test that evaluates for three organisms at once, *T. vaginalis, G. vaginalis, and C. albicans*. When testing for TV, Affirm has improved sensitivity (92%) and specificity (99%) compared to culture techniques. The test takes 45 minutes to complete with less than 5

minutes of hands-on time. Results are color coded for easy reading. The Affirm test is also only available for the testing of vaginal secretions and not approved for urethral swab testing. Similarly to OSOM, Affirm tests for the presence of DNA and not for live organism, leading to the same possibility of false positives in recently treated patients. In populations where the prevalence of TV is low, the positive predictive value of these tests is lowered leading to the possibility of increased false positive results.

Nucleic acid amplification tests (NAATs) are highly sensitive and specific assays for the detection of organisms. The APTIMA Combo2 assay (manufactured by Gen-Probe, Inc) was developed for the detection of TV in vaginal and endocervical swabs and female urine. The test has sensitivity ranging from 74–98% and specificity of 87–98%, it is 20% more sensitive than culture.[69,71] Similarly to the point of care tests, NAATs do not differentiate if the parasite is living. NAATs test for the presence of specific DNA targets, these strands of DNA may exist after the parasite has already been successfully treated. Research is ongoing to determine the length of time after treatment for the vagina to have completely cleared the remnant DNA. If a patient is retested prior to the clearance of the killed parasite it is possible that they will test positive. This method of testing is usually done in batches and is dependent on the amount of women to be tested as to the frequency of the testing. Results can take as little as 1 day in high volume locations, or up to 2 weeks.

Approved research laboratories have the ability to create their own primer targets for the detection of TV using polymerase chain reaction (PCR). This

allows them to test for their own specific targets. Laboratories that create their own PCR test for TV have the potential for tests that are overly sensitive, as only small amounts of the amplicon from contamination can result in a false positive test result.

TV in Men

The majority of laboratory testing methods currently available are only FDA-approved for testing in women. Microscopy is a possible testing method for males but the sensitivity is low. Culture can be used and it can be inoculated with several specimens collected from an individual in an attempt to increase the likelihood of detecting the infection. Unfortunately, this method still has low sensitivity in men. The point-of-care tests are not approved for use with male urethral samples. There are also no FDA approved NAATs for male specimens. Individual laboratories can conduct validation studies for the testing of urethral swabs and urine from men on the system that is approved for women. Due to the lack of optimal testing, men are usually being treated for TV only if they have a positive female partner. Symptomology in men is characterized as non-gonococcal urethritis. First line treatment for non-gonococcal urethritis, however, does not include coverage for TV. A study conducted in Malawi (2003)[39] suggested that treatment for TV should be given for non-gonococcal urethritis only if symptoms persist after first line treatment has been administered. An article by Sena, et al 2012, corroborates these findings in that men with non-gonococcal urethritis only 13% were positive for TV.[72]

Treatment

There are different options for treatment of TV and successful treatment can be dependent on other co-infections. In the US, the most common treatment is a single dose of 2 grams MTZ; other options include tinidazole and seconidazole. The other commonly used dosage is a 7 day course of 500mg BID MTZ; this is the same dose used to treat BV. Metronidazole is considered safe to use in all stages of pregnancy and it is recommended to withhold breastfeeding during treatment and up to 24 hours after treatment completion but it is not yet approved by the Federal Drug Administration for use in pregnant women.[73,74]

Partner Treatment

Expedited partner treatment (EPT) is when the partners of patients who are known to be positive for an STI are treated for that infection without being required to undergo medical evaluation and testing. EPT is usually performed by giving medication to the patient to deliver to their sexual partners; this is known as patient delivered partner treatment (PDPT). PDPT is allowable for the treatment of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in a majority of states. The Centers of Disease Control and Prevention, however, has yet to recommend its use for the treatment of partners of those infected with TV. Gatski et al. presented evidence that, among HIV+ women, the use of PDPT was an acceptable partner treatment method.[75] Previous randomized control studies using PDPT for the treatment of TV have found conflicting results efficacy with respect to HIV negative women. [11,29]

Treatment failure

The single dose treatment may not clear the infection; there is a 16.8% recurrence rate among women treated with single dose therapy. In comparison, the multi-dose treatment of 500mg MTZ BID for 7 days has a recurrence rate that is roughly half of the single dose.[21]

In studies that have performed test of cure visits on treated women the reinfections rates of TV are high, up to 18% among HIV positive women.[76] Clinics do not routinely perform test of cure visits; they will only retest/retreat if a patient complains about new or ongoing symptoms. Thus, there remains a possibility that after treatment the TV may be weakened and not completely cured; this could lead to an asymptomatic infection that could persist for years. In populations with known high prevalence of TV it is recommended that women be re-tested post treatment.

Co-infections of TV and BV, diagnosed by Gram stain, are common, 66% in a population of HIV+ women.[19] In a study by Gatski et al., co-infection of BV interfered with single dose MTZ treatment of TV, resulting in a higher post-treatment infection rate, 24% for co-infection versus 6% for TV alone. They suggest that because BV is often underdiagnosed in the presence on TV, that HIV+ women should be treated with the MTZ multidose regimen to cover both infections. The current treatment for BV, a 7 day course of 500mg BID MTZ, is sufficient for effective treatment of TV. If a woman is misdiagnosed with BV only, the treatment should not cause her harm and will most likely clear her of the TV infection. The problem with the misdiagnosis is that neither the woman nor her

sexual partner(s) will be aware they are/were infected with an STI. There is a high probability that the woman will be re-infected if she continues to have unprotected sex with any of her previous sexual partners as the majority of males with a positive TV sex partner are also positive.[77] If a woman is not correctly treated for both infections there is a high possibility that she will remain positively infected.

Misclassification Issues

Each type of diagnostic testing, as described above, can result in the misclassification of an individual as either false-negative or false-positive. None of the above tests have perfect sensitivity and specificity and so there remains the possibility that an individual will receive the wrong result. Wet mounts and culture methods both are at risk for classifying individuals as false negatives for TV detection due to their relatively low sensitivities and possible technician error, as compared to NAATs. There is the possibility of false positives due to remnant DNA or antigens if a patient is re-tested too soon after treatment completion.[78] To avoid the misclassifying of individuals as false positive, follow-up visits should be scheduled at more than 3 weeks after the completion of treatment.

Retention Issues

The principal issues with conducting treatment and TOC visits are the associated costs, the uncommon nature of TOC visits outside of studies, and the difficultly associated with retention of individuals. The populations with the highest prevalence of TV are also the groups that have the highest treatment failure rates. In the US, these populations consist primarily of low-income African-

American women. It can be difficult to ensure that the women return to the clinic for retesting after treatment as part of a study, since women who are no longer experiencing symptoms may not be inclined to undergo further evaluation. Many of the women of low socioeconomic status may find it difficult to take time off work to attend clinics that do not have night and weekend hours. In the clinic where this research was conducted, children are not allowed and women who are unable to find childcare are often not able to return to the clinic for further testing. All of these barriers make it increasingly difficult for women to attend clinic for retesting. Women that are unable to return to the clinic for retesting or retreatment also are at an increased risk of having persistent TV.

Analysis Issues

There are several obstacles to determine if a treatment failure has occurred. As mentioned previously under treatment failure, there are several co-infections and medications that may influence the likelihood of a re-test positive. In the analysis phase, these potential confounders must be examined to determine their involvement in interfering with treatment.

Poor compliance with medication must also be considered a factor that influences a positive result on retreatment. The common dose of 2g MTZ can cause vomiting and thus an inadequate dosage is received. The smaller dosages that are taken over several days have less chance of induced vomiting but a higher rate of non-compliance. Patients whose symptoms are alleviated after a few days of medication may not complete the entire course of prescribed medication. In either of these cases, it is a lack of compliance that has caused

the treatment failure and the patient may be retreated with the same medication with counseling to improve completion. Medication reactions and compliance need to be assessed when performing the analysis.

A true treatment failure can be difficult to differentiate from possible re-exposure among women who report vaginal sexual intercourse between the time initial treatment was given and the test of cure visit. Women who are re-exposed to a baseline sexual partner may test positive at a test of cure visit but their current infection may not be due to treatment failure but due to reinfection. To determine if the sexual exposure is a possibility the results of partner completion treatment must also be assessed. Effective partner treatment and abstaining from unprotected sex until treatment completion will greatly decrease the risk of reinfection. As such, sexual exposure and partner treatment data are essential to collect from all TV positive women and be included in the data analysis.

Origins of repeat infections

There are several possible routes that would cause a patient to test positive at a retesting visit. The medication may not have been sufficient for a complete eradication of the parasite, in which case they should be treated with a more intensive treatment regimen. Metronidazole-resistant TV is rare, around 3%,[79,80] but barring other modes of re-infection, it may be advisable to send the specimen for resistance testing, if after completion of the more intensive treatment, the woman remains positive. Specimens that are resistant to MTZ may be susceptible to other medications, i.e. tinidazole, or higher doses of MTZ.

A possibility for reinfection is sexual exposure from a baseline sexual partner. Many clinics rely on the infected patient to inform their sexual contacts about their possibility of exposure. Sexual partners are sometimes not told or they choose to not seek out treatment, putting the initial patient at risk for reinfection. Some clinicians have instituted expedited partner treatment, where the patient is given medication to distribute to their sexual partners.[81] Expedited partner treatment, as described in the above section, has the potential to decrease the likelihood of TV reinfection but has not been found to have a significant impact on a woman's re-test positive.[82]

Another possibility is infection from a new sexual partner who is currently infected with TV. With TV having a high population prevalence in many communities, it is possible that unprotected sex with a new sexual partner, after treatment completion, could lead to a new infection of TV. Since genotyping TV only occurs in research settings and is not part of clinical practice it makes it difficult to ascertain if the positive re-test is due to treatment failure, new exposure, or re-exposure. Even if the genotype is tested and found to be the same between baseline and follow-up, it is still difficult to determine if the infection is treatment failure or re-exposure. A way to differentiate the two is by assessing the patient's sexual exposures since treatment. It can be difficult to assess as patients may be reluctant to provide details of their sexual exposures in an in-person interview. Determining the a reliable method of sexual diary data collection will aid in making the determination if a repeat positive TV test is due to treatment failure or sexual exposure.

Smoking and TV acquisition

Smoking has been associated with several biological changes in the female genital tract. Nicotine, cotinine, and other chemicals associated with tobacco and their metabolites have been detected the cervical mucus. [83,84] It is hypothesized that the accumulation of amines from nicotine and cotinine are responsible for changes in the vaginal environment. These chemicals and their metabolites are basic compounds that raise the pH of the vaginal mucosa, which will favor the overgrowth of bacteria associated with BV. A significant dose-response relationship with the number of cigarettes smoked per day and BV has been established.[85,86]

Wilson et al. showed a decrease in the mean estrogen levels among smokers compared to non-smokers. Decreased estrogen levels at the beginning of the menstrual cycle have been shown to be associated with abnormal vaginal flora development.[87-89]A decrease in Langerhans cells, the most prominent type of antigen-presenting cells in the normal cervical epithelium, is significantly associated with cigarette smoking.[90,91] The loss of Langerhans cells may assist in the persistence of infections and increase the likelihood of neoplastic transformation. In a large cross-sectional survey of Nordic women there was a higher prevalence self-reported ever having TV among smokers compared to non-smokers.[92]

Most studies that have examined the interaction between TV and BV/abnormal vaginal flora have been cross-sectional or have been unable to determine which came first.[12,93-95] Many of these studies have shown that the

presence of BV increases the likelihood of infection with TV. Rathod et al performed a longitudinal study on women without TV at baseline and followed them at 3 and 6 month visits.[96] The authors found a 4- to 9- fold increase in the risk of TV infection among women who had had abnormal vaginal flora in the preceding 3 month time-span. This suggests that an increase in abnormal vaginal flora places a woman at higher risk of becoming infected with TV compared to women with normal vaginal flora.

Reporting of sexual behaviors

Assessment of sexual exposures is necessary for the evaluation of health issues, such as those associated with sexually transmitted infections (STI), pregnancy, and overall sexual health. Certain STIs have low drug resistance and yet the repeat infection/treatment failure rate is unusually high. For example, *Trichomonas vaginalis* (TV) has resistance rates as low as 3%[80,97] but repeat infection rates can be as high as 36% among HIV positive women,[11,12,21,76,98,99] it is vital to ascertain if the repeat positive is due to treatment failure, exposure to a new sexual partner, or caused by re-exposure to a previous infected sexual partner.[3,4,10,14-17] While genotyping can be helpful, it cannot differentiate between treatment failure and re-exposure.

After the possibility of treatment non-compliance, organism resistance, and treatment failure due to other host factors/medications have been ruled out, the remaining route is a new sexual exposure. The only way to determine the difference is through the patient's sexual history post-diagnosis and treatment. Currently, this method has its own challenges and limitations.

Data collection of sexual behaviors usually relies on self-report. Often these self-reports occur through the use of retrospective surveys, typically 1-3 months. There is a potential for recall errors that can lead to under- or over-reporting of risky behaviors. Shorter time periods of recall may minimize the potential for recall bias.

A daily or frequent collection of sexual behavior information may place undue burdens of time, effort, and expense both on the participant and the study personnel. A longer time between data collection may introduce several potential biases: participants may have systematic differences in their memories of sexual exposure (recall bias), they may include events that do not occur within the study time period (telescoping effect), or under- or over-report their sexual behavior based on their perceived social status (social desirability bias).[100,101] Specific details may be difficult to recall, e.g. amount of condom usage if inconsistent.

There are several different media through which sexual behavior information can be collected. Traditionally, data have been recorded using a paper survey. [102-106] Paper surveys have several disadvantages. Participants may feel uncomfortable recording sensitive information that may become accessible to others. Also, there is no way to ensure the timely completion of the paper survey, i.e., whether the surveys were completed daily or en masse just prior to study end. Electronic forms of data collection can overcome these disadvantages. Electronic diaries can be encoded, password protected and a time stamp can be applied for every data entry. Method of collection via Audio Computer-Assisted Self-Interview (ACASI) has been shown to be superior to

face-to-face interviewer (FTFI) collected.[107] Having subjects enter their sensitive information directly into the computer, without intervention from the interviewer, has led to a decrease in social desirability bias. Despite decades of assessing sexual exposures, there is as yet no standard in place for the collection of sexual behavior data for prospective studies. This review compiles methods and results from studies that have compared data collection methods which encompass the same participant calendar time.

HYPOTHESIS AND RESEARCH QUESTIONS

Study 1: Manuscript 1

Does ART usage interfere with MTZ medication for the treatment of TV in HIV infected women?

Aim 1: To describe the demographic characteristics of HIV+ women on ART and those not on ART.

Hypothesis: Women on ART will be different demographically from women who are not on ART.

Aim 2: To determine if TV retest positive is associated with ART usage.

Hypothesis: Women on ART will have a higher rate of re-test positive than women not on ART.

Aim 3: To determine if TV retest positive among women on ART is dependent on dose of MTZ.

Hypothesis: Women on ART and receiving the single dose of MTZ will have a higher rate of re-test positive compared to women on ART who received the multidose of MTZ.

Study 2: Manuscript 2

Does smoking increase retest positives of TV among HIV-positive women who have been previously treated with MTZ?

Aim 1: To describe the demographic characteristics of HIV+ women who smoke

Hypothesis: Women who smoke will be different demographically from women who do not smoke.

Aim 2: To determine if smoking increases the risk of a TV retest positive.

Hypothesis: Women who smoke will be more likely to test positive at a follow-up visit than women who do not smoke.

Study 3: Manuscript 3

Are recall methods of data collection regarding sexual activity similar to more frequent prospective methods?

Aim 1: To determine the data collection methods used in the comparison of prospective and recall assessment.

Aim 2: To determine the length of prospective and recall data collection and the time between the collections.

Aim 3: To determine if the recall method of data collection is similar to prospective measures assessing over- and under-reporting of sexual behaviors.

STUDY 1: ART INTERACTIONS WITH METRONIDAZOLE TREATMENT

Project Summary: Manuscript 1

In a study among HIV+/TV+ women in Africa, Balkus et al presented evidence that antiretroviral medication (ART), in particular NNRTIs, interacts with MTZ treatment.[108]They found that the 2 g single dose of MTZ had a lower success rate among women who were receiving ART. They suggested that the mechanism for action was that nevirapine and MTZ are metabolized in the liver by enzymes in the cytochrome P450 system. Nevirapine up-regulates the cytochrome P450 system and could increase the clearance of MTZ. To compare

our findings to Balkus et al., a secondary data analysis of TV treatment outcomes for all women enrolled in a previously published study was conducted.

This study has several improvements over the Balkus article. The population is expanded to include many types of ART, not just nevirapine. Shorter follow-up time is used to minimize the likelihood of a new infection. Sexual re-exposure was tracked with a retrospective survey covering the time between treatment and the TOC visit. BV co-infection was analyzed for possible confounding. Because this study used data from a previously reported randomized control it also has the advantage of examining the interaction of ART with single and multi-dose MTZ.

Objectives: Manuscript 1

The purpose of this study is to examine the influence of ART on the success of MTZ treatment of TV among HIV+ women in the U.S. and to determine if ART could be a factor in TV retest positive.

Materials and Methods: Manuscript 1

Study population: Manuscript 1

Data was collected during a previously reported randomized control trial for the treatment of TV amongst HIV+ women.[21] HIV+ women attending public HIV outpatient clinics in New Orleans, Louisiana; Houston, Texas; or Jackson, Mississippi from May 2006 to July 2009 were tested for the presence of TV by culture as a standard of care test during their routine gynecological examination.

Women were eligible for enrollment if they were HIV+ (confirmed by Western blot), ≥18 years of age, English speaking, TV positive by culture, willing

to take MTZ and to refrain from drinking alcohol for the length of MTZ treatment and for 24 hours post treatment completion. Women were excluded if they were pregnant, breastfeeding, incarcerated, taking disulfam, or were treated with MTZ within the previous 14 days.

At the enrollment visit women completed an audio computer assisted self-interview (ACASI) assessing their demographic information, current medications including ART, substance use, douching practices, current smoking status and recent sexual history. ART was elicited by abstracting prescribed medication from the medical records and then confirming with the patient's self-report of whether or not they were taking the medicine. Patients were asked which HIV medications they used by trained study staff personnel using a chart with pictures of medications available during the study time period (May 2006 to July 2009). When ART status was discrepant between medical records and patient-report, the patient-report was used. ART adherence was assessed by asking the women "Yesterday, did you take your (medication name) as prescribed?" BV was diagnosed in women who had a Nugent score ≥ 7. CD4 cell count and plasma viral load were abstracted from the woman's most recent lab results in the clinic chart.

Eligible women who agreed to participate in the study were randomized to receive either 2 g single dose MTZ or 500 mg MTZ twice-daily for 7 days. Women in both treatment arms were given 2 g single dose MTZ to deliver to their sexual partner(s), up to 4.

A test of cure (TOC) visit was conducted 6-12 days post treatment completion and a follow-up visit was conducted at 3 months post treatment completion.[20,109] Women were tested for the presence of TV by culture and completed a survey assessing possible sexual re-exposures, treatment compliance, and social and behavioral activities. Sexual re-exposure was defined as having unprotected sex with a baseline partner prior to completion of medication by participant and/or partner.

Laboratory procedures: Manuscript 1

Eligible women were tested for the presence of TV at screening and, if enrolled, at their TOC visit. TV positivity was determined by use of the InPouch culture (InPouch culture, Biomed Diagnostics, White City, Oregon). Vaginal swabs were either self- or provider-collected at screening and self-collected at TOC, as self-collected swabs have similar predictive values for TV diagnosis.[110] The vaginal swabs were placed into the InPouch following the manufacturer's protocol. Pouches were examined under the microscope for TV immediately upon receipt. The pouches were incubated at 37° C and staff obtained three daily readings within a five-day period. A diagnosis of TV was made after the first positive pouch reading. Three negative pouch readings were require to consider the woman TV-negative.

The presence of BV was determined by collecting a vaginal swab that was rolled onto a slide three times. The slide was later sent to a laboratory at Louisiana State University for Gram staining and Nugent scoring.[59]

Statistical analysis: Manuscript 1

Statistical analysis was conducted using SAS 9.2. HIV + women reporting ART usage at baseline were compared to women not using ART. Covariates collected at baseline that were assessed for possible confounding included Race (Black/Non-Black), Unemployment (Yes/No), Education (Less than high school/ high school or more), Cohabitating (Yes/No), Smoking (Yes/No), Alcohol (Yes/No) , Douching (Yes/No), Co-infected with BV (Yes/No), Number of sex partners , Sexual re-exposure (Yes/No), CD4 cell count ≤ 200/mm3 (Yes/No), and HIV viral load >10,000 copies (Yes/No). BV status, CD4 cell count, and plasma viral load levels were categorized as dichotomous variables for above or below the cut point, BV Nugent score ≥7, CD4 cell count ≤ 200/mm3 , and plasma viral load >10,000 copies. Potential confounders were evaluated by a 10% change in the beta estimate and their association with the exposure and outcome. Only confounders that caused a significant change were included. Relative risk with a 95% confidence interval by chi-square and log-binomial regression was calculated to assess the relationship between ART usage and repeat TV infection at TOC. The relationship between exposure and outcome was also examined after stratification on the MTZ dose (single vs multi) taken.

Exposure was defined as use of ART prior to baseline visit, as determined by patient interview and medical chart review. If any discrepancy between the two records occurred, the patient's interview was used as fact. Patients not on ART at time of visit and who received a prescription at that visit were not considered to be taking ART. To be considered on ART the patients had to report

consistent use, which was defined as a positive response to patient's taking her pills the day before. Outcome was defined as a positive detection of TV at 1 week post treatment completion test of cure visit. Testing method was either positive wet mount or culture.

The original RCT and secondary data analyses (Tulane University Biomedical IRB, New Orleans, LA 14-563551E) were approved by the institutional review boards for the individual study sites and written informed consent was obtained from all women prior to randomization and treatment.

Power Calculation: Manuscript 1

It was assumed that those not on ART had a lower rate of retest positive, with a proportion of 0.06. Assuming an alpha of 0.05 and the set sample size of 226, the below Table1 details the relative risk and related power. To achieve 80% there will need to be a relative risk greater than 3.2. In the Balkus study, they saw a 2.88 (95% CI 1.32, 6.30) increase in the risk of persistent TV compared to those not using ART.

Table 1: Power calculation for ART and TV

Computed Power	
Relative Risk	Power
2.5	0.52
2.6	0.567
2.7	0.613
2.8	0.656
2.9	0.697
3	0.736
3.1	0.771
3.2	0.804
3.3	0.833
3.4	0.859

Limitations: Manuscript 1

Sample size was limited to the number of women who met the inclusion criteria for analysis, which in this data set is 226. Wet mount and culture was used to determine TV positivity. NAAT would have been more sensitive, but would be inappropriate for a 1 week follow-up. NAAT may be falsely positive before 3 weeks post treatment due to remnant DNA that has not been cleared from the vagina.[78] ART consistent use was defined by patient self-report which may be subject to social desirability bias. No tests were used to assess amount of ART in the patients' systems. A post hoc power analysis reveals only 60% power was achieved for the whole cohort and only 54% power in the single dose arm and 13% power in the multidose arm for the stratified analysis.

Results

Out of the 270 HIV+ and TV+ women enrolled in the RCT, 226 attended their test of cure visit and had complete data for analysis. Table 2 presents demographic information on enrolled women. The majority of women were African-American (92%) and unemployed (70%). Over half of the women (65% n=146) were taking ART at their baseline visit and of those on ART, 93.8% report previous day compliance with medication at baseline. Overall duration of ART was not assessed. Less than a third of the women were married or cohabiting with a current partner and 40% did not graduate high school. Nearly half the women reported being a regular smoker (44%) and over a third reported drinking alcohol in the last week (38%). A majority of the women had BV (67%) as diagnosed by Nugent score of ≥7. Similarly, 67% of women reported that they

douched in the last 30 days. In the three months prior to enrollment 76% of women reported having 1 or more sexual partners (either male or female) in the past 3 months. Demographic information did not differ based of whether the woman was on ART or not on ART at baseline.

Women who were on ART at baseline were more likely to have CD4 cell counts below 200/mm^3 (23% compared to 17% p-value=.003). A CD4 cell count ≤ 200/mm^3 was only associated with a positive TOC in those women receiving the multidose treatment. Viral load was not associated with a positive TOC in either treatment arm. Viral loads above 10,000 copies per mm were common in women not on ART (50%) when compared to on ART (27%) p-value 0.0007.

The distribution of types of ART taken by women at baseline is shown in Table 3. Of women currently taking ART nearly all were on a nucleoside reverse transcriptase inhibitor (NRTI) (95%), a majority were on a protease inhibitor (PI) (61%), with about one third on a non-nucleoside reverse transcriptase inhibitor (NNRTI) (36%), and nearly none taking other types of HIV medication (3%).

The relative risk of treatment failure was 2.63 (95% CI 1.04-6.63) for women taking ART compared to those not taking ART, p-value=0.03. When examined by treatment arm and ART usage, the association was only found in the single-dose arm (RR 3.16 95%CI 0.99-10.08 p-value= 0.05) and not in the multi-dose arm (RR 1.97 95%CI 0.43-9.03 p-value=0.39), Table 4.

BV was equally distributed among the two groups of women on ART (67.8%) and not on ART (65.0%) p-value=0.67 overall and was equally distributed when examined by treatment arm. A plasma viral load greater than

10,000 was associated with the women being prescribed ART (p=0.0007) but was not independently associated with the woman experiencing treatment failure (p=0.600). When stratified by treatment arm plasma viral load remained unassociated with treatment failure, single dose (p=0.180) and 7 day dose (p=0.106). CD4 cell count was not associated with treatment failure overall (p= 0.295) but when stratified by treatment arm it was significant in the 7 day dose (p=0.033) and not in the single dose (p=1.00). However, when included in an adjusted model it did not significantly change the effect estimate and was thus excluded.

Another analysis was conducted to examine if the association was still present in treatment regimes that didn't contain a NNRTI. Given that only 36% of women were currently prescribed a NNRTI we did not find any significant differences between models that examined any ART usage or models that limited analysis to women not receiving NNRTI.

Sexual re-exposure was not included in the final model for analysis, as none of the women who were TV+ at TOC met the criteria for sexual re-exposure and it was equally distributed between ART statuses. Out of 226 women seen for their test of cure visit, 5 reported having unprotected vaginal intercourse prior to mutual treatment completion, none of these women were positive for TV at TOC. No women reported having unprotected sex, sharing unwashed sex toys, or mutual masturbation with baseline female partners.

Table 2: Baseline characteristics of HIV+/TV+ women by ART status (N=226)

	ART Yes n=146	ART No n=80	p-value*
African-American	133 (91.1%)	74 (92.5%)	0.74
Unemployed	108 (73.9%)	50 (62.5%)	0.10
Did not graduate high school	59 (40.41%)	32 (40.0%)	0.95
Married or cohabitating	34 (23.3%)	25 (31.3%)	0.19
Regularly smokes cigarettes	61 (41.8%)	37 (46.2%)	0.52
Drank alcohol in the past week	52 (35.6%)	34 (42.5%)	0.31
Has vaginally douched in the last 30 days	101 (69.2%)	52 (65.0%)	0.52
BV	99 (67.8%)	52 (65.0%)	0.67
≥1 sex partner in past 3 months	106 (72.6%)	67 (83.7%)	0.06
Multi-day dose MTZ**	73 (50.0%)	41 (51.3%)	0.86
Sexual Re-exposure	2 (1.3%)	3 (3.7%)	0.57
CD4 cell count ≤ 200/mm3	53 (23.4%)	14 (17.5%)	0.003
Viral load >10,000 copies	40 (27.4%)	40 (50%)	0.0007

*p-values obtained by chi-square analysis

**Patient randomized to receive the multi-day dose of 500mg BID for 7 days vs the single dose of 2g MTZ

Table 3: Types of ART prescribed among HIV+ women currently receiving any ART

ART Class	n=146
NRTI	138 (95%)
PI	89 (61%)
NNRTI	53 (36%)
Other	4 (3%)

Table 4: Post treatment TV infection rates at test-of-cure by ART status and MTZ treatment arm among HIV+ women (N=226)

	Percentage overall persistent infection rate TV+ (n)	Percentage persistent infection rate TV+ on ART	Percentage persistent infection rate TV+ not on ART	RR (95% C.I.)	p-value
Test of Cure	12.8 (29/226)	16.4 (24/146)	6.3 (5/80)	2.63 (1.04-6.63)	0.036
Single dose	17.9 (20/112)	23.3 (17/73)	7.7 (3/39)	3.16 (0.99-10.08)	0.052
Multiday dose	7.9 (9/114)	9.6 (7/73)	4.8 (2/41)	1.97 (0.43-9.03)	0.385

Discussion

Both the parent study and the secondary data analysis of the interaction of BV on treatment, found that in HIV+ women the multi dose therapy of MTZ is superior to the standard of care treatment of single dose MTZ. This current data analysis uses the information gathered from the RCT and the knowledge gained from the BV study to evaluate the possible interaction of ART on the treatment of TV. Our study addresses some of the limitations that were present in the Balkus et al study. Our TOC visit was 7 days post treatment completion whereas their assessment was up to 60 days after treatment. The longer follow-up and their study population, female sex workers, may explain why their cohort had 50% sexual exposure compared to 2% in our cohort. HIV+ women receiving ART in the Balkus cohort were prescribed nevirapine containing regimens, whereas in our cohort the vast majority of woman on a NNRTI were prescribed efavirenz. Only one woman received nevirapine. Our results demonstrate the ART effect is not specific to treatment regimens containing nevirapine.

There are a few limitations to this secondary data analysis. Because the study was not initially designed to address the possible interaction of ART on MTZ treatment for TV, there is a lack of statistical power. A post hoc power analysis reveals we only achieved only 60% power for the whole cohort and only 54% power in the single dose arm and 13% power in the multidose arm for the stratified analysis, none-the-less, we did find statistical association. Another limitation is the use of culture rather than nucleic acid amplification testing (NAAT). We used the InPouch culture to determine TV positivity, while InPouch is more sensitive than wet mount microscopy (which was used in Balkus et. Al.

study), it is not as sensitive NAAT. It is possible that attenuated TV infections were missed at TOC.

This study can't establish causality as it lacks experimentation and it is possible that ART is a marker of some other biological factor that interferes with MTZ treatment of TV. The possibility remains that the ART regimens that lack nevirapine, also have an effect on the metabolism of MTZ. It is recommended that to further elucidate the interaction of ART and MTZ that pharmacokinetic studies be conducted.

Our study results show that ART is likely to interact with MTZ for the treatment of TV. This interaction occurs in the single 2 g MTZ dose and may occur in the multidose MTZ, however, the sample size was insufficient to detect the interaction. In conclusion, our data suggests that the multidose of MTZ may be superior to single dose MTZ in the treatment of TV amongst HIV+ women receiving ART.

STUDY 2: SMOKING INTERACTIONS WITH TV ACQUISITION

Project Summary: Manuscript 2

Smoking has been shown to alter the normal vaginal flora and a dose response between amount of cigarettes smoked per day and an increased risk for contracting bacterial vaginosis (BV) has been established[111]. Both of these may increase a woman's susceptibility to sexual transmitted infections, such as TV.

Objectives: Manuscript 2

This study was conducted to assess the role of smoking in the retest positive of *Trichomonas vaginalis* (TV) among HIV+ women.

Materials and Methods: Manuscript 2

Study population: Manuscript 2

Data for this study was from the same cohort that was used in Study 1 above. The follow-up data for this study was collected 3 months after the baseline visit. Women were administered a survey similar to the one given at baseline and at the TOC visit, that asked them about the last 3 months of their lifestyle and sexual behavior. Only women who attended the TOC visit and were negative for TV by wet mount and/or culture at that time were included in this analysis.

Laboratory Procedure: Manuscript 2

Laboratory procedures were the same as in Study 1.

Statistical analysis: Manuscript 2

Statistical analysis was conducted using SAS 9.2. Covariates collected at baseline to be assessed for possible confounding included race, unemployment, education (less than high school/ high school or more), cohabitating, ART, alcohol, douching in the last 3 months, co-infected with BV , number of sex partners, sexual re-exposure, CD4 cell count ≤ 200/mm3, HIV viral load >10,000 copies. BV status, CD4 cell count, and plasma viral load levels were categorized as dichotomous variables for above or below the cut point, BV Nugent score ≥7, CD4 cell count ≤ 200/mm3 , and plasma viral load >10,000 copies. Relative risk with a 95% confidence interval was calculated by chi-square and log-binomial regression to assess the relationship between smoking and TV infection at 3 month follow-up. The relationship between exposure and outcome was also be examined after stratification on the MTZ dose (single vs multi) taken. Since there are several levels of smoking being assessed, a Cochran-Armitage trend test was also performed.

Exposure was defined as cigarette smoking at baseline visit. Cigarette smoking and amount of cigarettes smoked was determined by patient interview and was classified as, no smoking, rarely smoke, regularly smoke less than a pack a day, and regularly smoke about a pack a day or more. Categories for rarely smoke and regularly smoke less than a pack a day were condensed into one group due to low numbers of outcomes. Outcome was defined as a positive detection of TV at 3 months post baseline. Testing method was either positive wet mount or InPouch culture (InPouch culture, Biomed Diagnostics, White City,

Oregon). The original RCT and secondary data analyses were approved by the institutional review boards for the individual study sites and written informed consent was obtained from all women prior to randomization and treatment.

Power Calculation: Manuscript 2

It was assumed that those that do not smoke will have a lower rate of repeat infection, with a proportion of 0.06. Assuming an alpha of 0.05 and the set sample size of 147, in which 63 don't smoke and 20 in highest smoking category, the below Table 5 details the relative risk and related power. To achieve 80% there needs to be a relative risk greater than 4.1. Previous studies examining the relationship between smoking and BV have found an OR of 2.3-3.0.[55]

Table 5: Power calculation for smoking and TV

Computed Power	
Relative Risk	Power
4	0.781
4.1	0.8
4.2	0.818
4.3	0.835
4.4	0.851
4.5	0.866
4.6	0.88
4.7	0.892
4.8	0.904
4.9	0.915
5	0.925

Limitations: Manuscript 2

Sample size was limited to the number of women who met the inclusion criteria for analysis, which in this data set is 147. Wet mount and culture was used to determine TV positivity; NAAT would have been a more sensitive testing

methodology and appropriate to use 3 months post treatment. Sexual re-exposure data was collected using a 3 month retrospective survey. This may be a significant limitation since the accuracy of a 3-month retrospective recall has not been assessed. A post hoc power analysis reveals that when comparing those women who smoke a pack a day or more to non-smokers only 70% power was achieved for the whole cohort and only 65% power in the single dose arm and 30% power in the multidose arm for the stratified analysis.

Results

270 women were enrolled in the original RCT, 226 attended their TOC visit and of those, 161 returned for the 3 month follow-up visit. A total of 143 women were TV negative at TOC and had complete data for analysis. Demographic information is presented in Table 6. The majority of women were African-American, taking ART, and unemployed. Over half were single, divorced, or widowed. A majority of the women reported douching in the last 30 days. Over 95% of the women are over the age of 25. Approximately 26% had viral loads >10,000 copies and 26% had CD4 cell count ≤ 200/mm3.

A significant trend value (0.004) was seen among those women who were unemployed however this relationship is more of a dichotomous relationship between smokers and non-smokers. There was no corresponding association detected between unemployment and TV infection. Even at the higher level of smoking there was no observed relationship with BV. Women at all levels of smoking were equally distributed between the MTZ single and multi-dose treatments. No other demographic characteristics are of note when examining smoking.

To determine which of the collected demographic characteristics were associated with TV, chi-square analyses were performed. Women under the age of 25 had higher rates of TV infection than older woman, which approached significance (p-value=0.079). Similarly, among those who reported 1 or more male sex partners there was a non-significant higher rate of TV positivity (p-value=0.092). There was no association observed with BV. The use of oral contraceptives in this population is low, 6.3%, and their use was not associated with TV.

The association between TV infection and smoking was assessed using the Cochrane-Armitage trend test examining the levels of smoking, Table 7. The overall rate of a positive TV result at 3 months was 16.7%. The rate varied depending on the initial dosage of MTZ, with a rate of 24.6% with single dose and 9.5% with multi-dose after 3 months post treatment. The univariate analysis between dose and TV infection was significant (p-value=0.007).

Each of the demographic characteristics was examined for possible confounding and none were found to cause a significant change in the estimate. Based on previous research and the observed association between MTZ dose and TV it was decided to examine the relationship between smoking and TV stratified on MTZ dose, Table 8. Non-smokers were used as the reference group. Using the entire population those in the higher level of smoking had a relative risk of 3.44 (95% CI 1.31-9.04, p-value=0.012). The lower level of smoking was not significantly associated with the TV outcome, p-values of 0.269.

In a stratified analysis by MTZ dose, those in the single dose MTZ arm and smoked a pack a day or more had a relative risk of 4.50 (95% CI 1.26-16.06, p-value= 0.021, and those that smoked less than pack a day had a relative risk od 2.65 (95% CI 0.81-8.68, p-value=0.108) when compared to non-smokers. The women in the multi-dose arm had a relative risk of 2.67 (95% CI 0.62-11.44, p-value=0.187) among those who smoked a pack a day or more and those that smoked less than pack a day had a relative risk of 0.36 (95% CI 0.04-3.23, p-value=0.359), compared to non-smokers.

In a multivariable model, where BV and ART use were also included as covariables, the higher level of smoking remains a significant risk overall and in the single dose arm. ART did not significantly alter the association between smoking and a positive TV test result, thus it was excluded from the final model.

Table 6: Characteristics of HIV+ women by smoking status (N=143)

	Smoking* %			
	Non-smoker n=59	<Pack/day n=64	≥Pack/day n=20	Cochran-Armitage Trend Test
Has douched in the last 30 days	59.3	46.9	65.0	0.430
Did not graduate high school	30.5	46.0	30.0	0.640
Drank alcohol in the past week	25.4	34.9	40.0	0.082
Under 25 years of age	3.4	4.7	5.0	0.351
Current hormonal birth control	6.8	6.3	5.0	0.393
BV	64.8	57.9	72.2	0.159
Unemployed	52.5	79.0	75.0	0.004
Single, divorced, or widowed	74.6	64.1	65.0	0.133
Inconsistent condom use	20.3	17.2	45.0	0.048
≥1 sex partner in past 3 months	13.8	9.5	5.0	0.122
Multi-day dose MTZ**	54.2	46.9	60.0	0.482
CD4 cell count ≤ 200/mm3	27.1	28.6	15.0	0.217
Viral load >10,000 copies	25.4	28.6	25.0	0.453
Antiretroviral therapy	72.9	71.9	55.0	0.106

Table 7: Test for trend of retest positive TV by increasing level of smoking status

	Smoking %			
	Non-smoker	<Pack/day	≥Pack/day	Cochran-Armitage Trend Test
Overall	10.2 (6/59)	17.2 (11/64)	35.0 (7/20)	0.007
Single dose	11.1 (3/27)	29.4 (10/34)	50.0 (4/8)	0.008
7-day dose	9.4 (3/32)	3.3 (1/30)	25.0 (3/12)	0.149

Table 8: Relative Risk of TV retest positive by smoking status and MTZ treatment dose

		RR	95% Confidence limits		p-value
			Lower	Upper	
Overall	<Pack/day	1.69	0.67	4.28	0.269
	≥Pack/day	3.44	1.31	9.04	0.012
Single dose	<Pack/day	2.65	0.81	8.68	0.108
	≥Pack/day	4.50	1.26	16.06	0.021
Multi dose	<Pack/day	0.36	0.04	3.23	0.359
	≥Pack/day	2.67	0.62	11.44	0.187

* Non-smokers used as reference group

Discussion

These results provide evidence that smoking a pack a day or more is associated with single dose MTZ retest positive in HIV+ women. In this study, women were known to be negative via culture at their ToC visit and denied any unprotected sexual exposure to a baseline partner. The vast majority of women reported no sexual contact with either baseline or new partners. Among the women that reported inconsistent condom usage over the preceding 3 months none of them were subsequently positive for TV. This decreases the likelihood that the TV infection observed at 3 months is due to new exposures.[76] Instead, it is possible that smoking is interfering with the MTZ treatment and thus enhancing the survival of the TV organism, such that TV is undetectable at the TOC visit but then multiplies after MTZ is cleared by the infected woman.

The relationship between smoking and TV was only significant at the higher level of smoking. However, there is also a significant trend with increasing levels of smoking; leading to the possibility that, smoking and increasing levels of smoking, interferes with successful treatment. Since the association is only observed in the single dose arm we posit that the longer length of treatment with multi-dose is able to overcome the effects of smoking on the vaginal flora and the women's innate immune response. In the multivariable analysis we observed that smoking a pack or more a day is a significant contributor to TV positivity after adjustment for other known risks. ART has been previously reported as possibly interfering with MTZ for the treatment of TV,[112] however this is not observed at the 3 month follow-up visit. This may be due to the limitation that ART

assessment was conducted at baseline. Prescribed ART changes were not accounted for during the follow-up period.

Previous research examining the relationship between TV and smoking was conducted using a cross sectional study design within a cohort of pregnant women.[113] Our study strengthens their results by examining TV occurrence as a prospective cohort. Since we followed the women's cure rates over time we are able to determine to a greater extent that smoking is indeed interfering with clearance of TV.

Most studies that have examined the interaction between TV and BV/abnormal vaginal flora have been cross-sectional or have been unable to determine which came first.[12,93-95] Many of these studies have shown that the presence of BV increases the likelihood of infection with TV. Rathod et al performed a longitudinal study on women without TV at baseline and followed them at 3 and 6 month visits.[96] The authors found a 4- to 9- fold increase in the risk of TV infection among women who had had abnormal vaginal flora in the preceding 3 month time-span. This suggests that an increase in abnormal vaginal flora places a woman at higher risk of becoming infected with TV compared to women with normal vaginal flora. Since BV was only assessed at baseline and not at the 3 month follow-up visit it is difficult to assess any effect that BV might present with smoking and the current TV infection.

A marginal association between oral contraceptive use and a lowered prevalence of TV colonization has been previously described. [113,114] The use of oral contraceptives in this population is lower than prior research, thus it is not

unexpected that there was no association with TV observed. Wet mount and culture was used to determine TV positivity, nucleic acid amplification tests (NAAT) would have been a more sensitive technique to detect treatment failure. However, NAAT is inappropriate to use at a 1 week ToC visit. At the 3 month visit NAAT may have detected additional cases of TV infection. Sexual re-exposure data was collected using a 3 month retrospective survey. This may be a limitation if the women do not accurately recall their type and frequency of sexual activities.

In previous analyses of this data set, the original RCT and secondary data analyses of the interactions of BV and ART, the multi-dose MTZ treatment was superior at successfully clearing TV infection compared to the single dose MTZ treatment. [21,94,115] Our findings in this study further indicate that that single dose is an ineffective treatment among HIV+ women, especially among those with asymptomatic BV, use of ART, and current smokers.

STUDY 3: REVIEW OF SEXUAL DIARY COLLECTION METHODS

Project Summary: Manuscript 3

There is no current standard for the collection of sexual activity data for prospective studies. This review evaluates studies that have compared types of sexual behavior collection methods which encompass the same participant calendar time, with the goals of reviewing their methods of data collection and assessing data accuracy.

Methods: Manuscript 3

A literature search was conducted for articles published through October 2014 using the following databases: Medline OVID, Medline EBSCO, and ISI Web of Science. The publication date was not limited, so as to include publications from all time points. Search terms included: (sex or sexual activity or sexual behavior or coital) and (diary or journal or account or log or record or monitor). The search terms used for the various methods of recording sexual behavior include: recall surveys of varying lengths (weekly, monthly, or multi-months) or event-based (same day recording of event); different methods of recording data with a live interviewer (paper surveys or phone calls); different methods of recording data, automated (on-line, smartphone application, text messaging, in-office computer assisted). All fields were searched in MEDLINE. Title, abstract, and keywords were searched in ISI Web of Science. The broad search terms were chosen so as to include as many publications as possible in the initial search.

Included articles were those that compared 2 or more methods of sexual behavior data recording and compared the same block of participant calendar time, i.e. compared the same 4 week period of data collected daily and then a recall survey covering the same 4 weeks. Studies were excluded if they compared different blocks of participant calendar time or were not written or translated into English.

Titles and then abstracts were reviewed for relevance, those that potentially met the inclusion and exclusion criteria were selected for further review. The methods of the articles were examined and articles that did not clearly state the methods used to collect sexual behavior information were excluded. The outcomes of interest reported in the articles were the differences between the reported number of sex acts (vaginal, oral, anal), number of sexual partners, and the consistency of condom use (if reported in the original study).

There is no "gold standard" for the collection of sexual behavior data and the articles included have differences in the methods they are comparing. The majority of articles consider the shorter evaluation time span as the valid measurement of sexual behaviors but there is no such consensus on the method of that collection.[116] Given that the daily reporting of sexual behavior is the shortest time span, the standard to be used for this manuscript will be daily data reporting using computer-based collection methods (to avoid reporting the data to a live person).

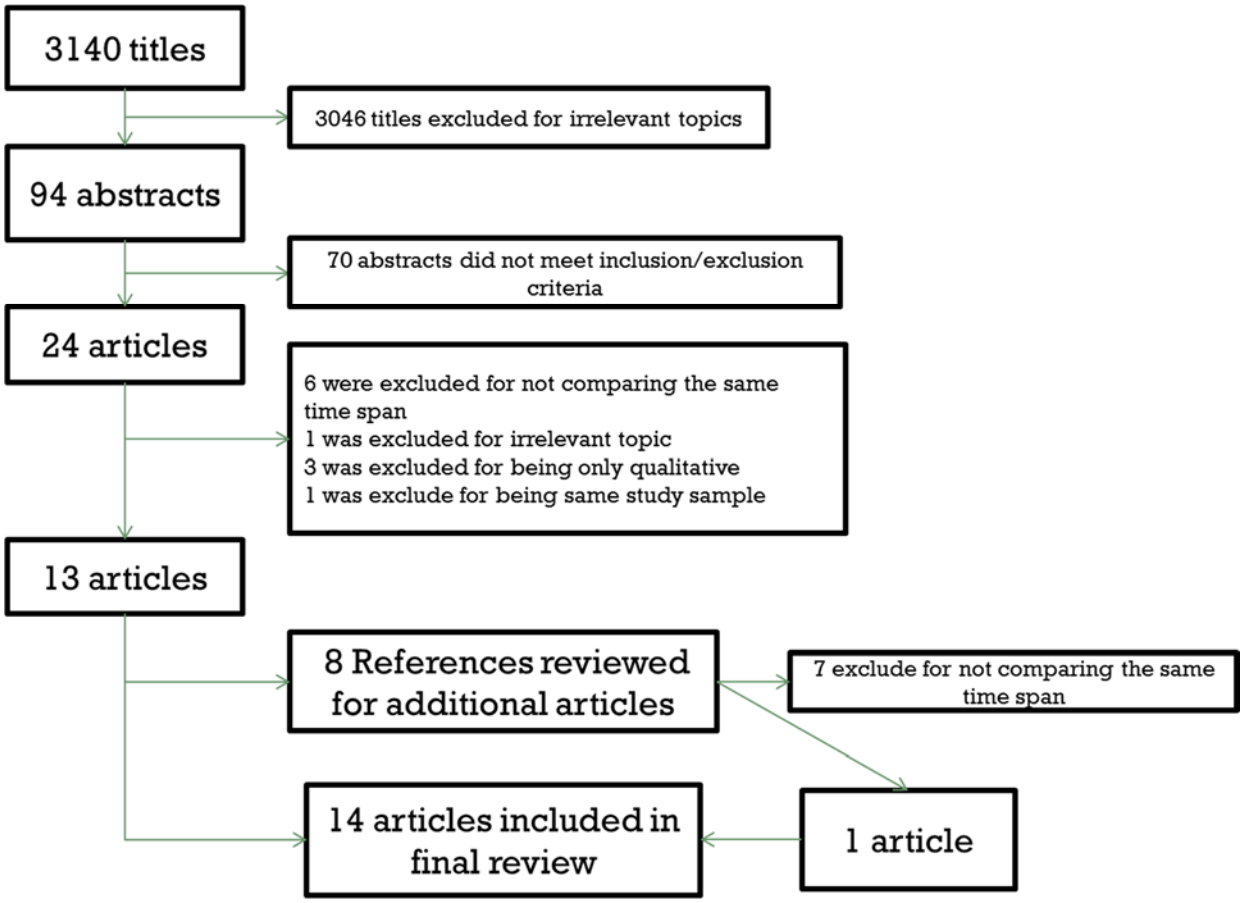
Limitations: Manuscript 3

This review was limited to articles published in English or articles that have been translated into English. There is the possibility of publication bias preventing the accessibility of data from studies that found no difference between different methods of sexual behavior data collection. There is no consistent "gold-standard" for method of collection; each study uses different methods to compare time spans and different collection techniques.

Results

Search results yielded 3140 titles for review. Of those, 3046 were excluded for irrelevant topics. The remaining 94 underwent abstract review for inclusion and exclusion criteria, 70 abstracts were rejected. Full article review was performed on 24 articles, of which 6 were excluded for not comparing the same time span, 1 was excluded for irrelevant topic, 3 were excluded for being only qualitative, and 1 was excluded for being a duplicate study sample. The 13 included articles had their references reviewed for additional articles; 8 articles appeared relevant based on title, but only 1 meet the inclusion/exclusion criteria. A total of 14 articles are included in this review.[105,106,117-128]

Figure 1 Flow diagram of article selection



The articles have a publication year range of 1998-2013. A majority (n=10) were carried out in the United States.[105,106,119-126] Other countries represented included Tanzania,[117] United Kingdom,[118] South Africa,[127] and Botswana.[128] The method of article selection and the corresponding results limits the current findings to countries and areas that are primarily English-speaking. The three studies conducted in non-English-speaking countries focused on populations who were either sex workers,[127] considered to be at high risk of being a sex worker, i.e. hospitality staff,[117] or were likely to patronize sex workers, i.e. soldiers.[128]

The mean number of participants enrolled in the studies is 133 with a range of 37-499. The articles have differing gender inclusions; 4 are male only,[118,120,123,128] 5 are female only,[105,117,121,126,127] and 5 enrolled both males and females.[106,119,122,124,125] With regards to sexuality, most (n=10) of the studies were conducted among heterosexuals,[105,106,117,121,122,124,126-128] 3 focus on men who have sex with men (MSM) only,[118,120,123] and 1 includes both heterosexual, men and women, and MSM.[125] Participants' age ranged from 14 to 44; the majority of studies included participants over 18 to mid-30s in age.

Prospective Data Collection Frequency and Duration of Reporting

While there is no standard length of time for the reporting of sexual behavior, it can be intuited that the shorter intervals, i.e. daily, will have greater accuracy.[116] In order to obtain an accurate assessment of an individual's risk, daily collection of data would have to continue for long enough time frames so as to be representative. This poses possible issues of participant and staff overburden. Researchers will often use retrospective surveys of recall length greater than 1 month to conserve resources. Comparing data collected over the same time span using a short interval prospective method and a longer interval recall method may provide insight into the accuracy of participant memory of sexual behavior.

Of the articles included in this review 11 had daily collection,[105,106,117-119,121-126] 1 used event-based collection,[127] and 2 used semiweekly, weekly, or biweekly as the shorter prospective intervals.[120,128] Amongst the studies that collected data daily, the time periods of the data submission to study personnel was

daily,[106,119,122,123,125,126] every 4 weeks,[118,127] or after 6 weeks.[121] The event-based collection was returned to investigators every 4 weeks.[127] Most common was daily (n=7) submission of reports. Only a few studies asked participants about their satisfaction with the prospective collection methods.[117,126] Of the 2 that reported participant satisfaction, Minnis et al found that automated telephone interviews were preferable among adolescents.[126] Allen et al, reported that there was increased monetary cost associated with those participants that received medium to intense interaction with study personnel but that the level of support did not affect the completion rates of the diary collection.[117] While many of the articles mentioned the concern of staff burden, there were no assessments reported regarding personnel time or monetary expenditures related to data management.

Length of Recall Data Collection

The recall length of time used for comparison ranged from 2 weeks to 3 months, with most (n=7) asking for recall of 4 weeks.[106,117-119,123,124,126] A majority of the studies (n=9) had less than a week between the end of the prospective data and the collection of the recall data.[117,118,121-128] This brief delay allows for there to be a lesser likelihood that other events that did not occur in the study period interfered with participant memory. One study[119] purposefully had 6-12 months window prior to interviewing participants; they chose the delay because they were interested in whether participants could remember events that occurred up to 12 months previously.

Data Collection methods

The method of data collection is also influential in the accuracy of the data collected. Participants respond with increased accuracy and higher degrees of comfort when answering sensitive questions using self-administered questionnaires (SAQs), either paper or computer, compared to conducting FTFIs.[106] A few studies employed more than 1 method of collection throughout their prospective phase.[121,122,124,126] Amongst the studies that only used one method, there were 6 that exclusively used paper SAQs,[105,106,117,118,127,128] 2 were web-based,[120,123] and 1 was email.[119] The remaining 5 studies evaluated more than one prospective method by assigning participants to either a paper SAQ or telephone collection method.[121,122,124,126]

For the recall methods, some studies used different methods of data collection than those that were used in the prospective method. There is the potential that the different method of assessing sexual behavior is the causative agent behind differences in the responses as opposed to simply memory errors. Only 4 used the same collection method, 2 paper SAQs[106,118] and the 2 web-based.[120,123] In one study,[119] subjects responded via email for prospective date collection then filled out a web-based survey for the recall portion; these two are highly similar methods of survey delivery and could be considered the same. There are 2 studies that did not discuss their method of recall collection.[121,127] Telephoned interviews were only used in 1 study, which also used telephones as an assigned method of prospective collection. SAQs were used in 4 studies; however, 1 of those was conducted in a group setting with the interviewer

reading aloud the questions.[128] Just 2 used FTFIs[105,117] and one of those also used SAQs for some participants. There were no included articles that made use of mobile phone applications (apps) for the either prospective or recall data collection. With ever expanding available technology, studies should explore the new data collection methods of text messaging and smart phone applications.

Comparison of Sexual Behavior Results

The main sexual behaviors under comparison are the number of heterosexual vaginal sex acts, condom use for vaginal sex, the number of anal sex acts, and condom use for anal sex. Several studies evaluated other sexual behaviors; however, these four were the most widely used amongst those included. The studies employed a variety of methods to compare the prospective and recall measures, i.e. kappa, comparison of means, discrepancy scores, or correlation. Though there is currently no 'gold standard' and each paper used slightly different methods, for the purposes of this review daily data collection methods were used as the standard for comparison.

When compared to the prospective collection, the recall methods under-reported (3 out of 14),[117,125,127] over-reported (n=4),[106,118,119,123] or found no significant difference (n=6);[105,120-122,124,128] one study found a significant difference but did not discuss its directionality.[126] Among the studies that reported no significant difference, 3 found non-significant over-reporting,[105,124,128] 1 was non-significant under-reported,[121] and 2 had no difference.[120,122] Overall, participants over-reported (n=7) their sexual behaviors more than they under-reported (n=4).

Of the over-reporting studies that showed a significant difference, sample sizes were less than 100, predominately male population, and 2 of the studies had long gaps, 6-12 months[119] and 1-3 months[106] between the prospective and recall data collection. The significant under-reporting studies include 1 whose population were female sex workers[127] and 1 that used FTFIs for the collection of recall data.[117]

Comparison of Results Stratified on Vaginal vs. Anal

Only 3 studies exclusively examined the differences in anal sex behaviors among MSM.[118,120,123] Two of the studies significantly over-reported the number of anal sex acts; they were similar in their length of assessment (4 weeks) and frequency (daily), one used paper SAQs[118] and the other used web-based surveys.[123] The third study found no significant difference in the mean responses.[120]

Excluding the one study that assessed vaginal and anal sex,[125] there are 10 studies that focused on vaginal sex.[105,106,117,119,121,122,124,126-128] There are an equal number of studies that under- or over-reported vaginal sex behaviors and 6 studies that found no difference. The under-reporting studies recruited female sex workers[127] or used FTFIs,[117] both of which may be prone deflate their numbers of sexual behavior due to social desirability bias. The 2 over-reporting studies are the ones discussed earlier with the longest time lag between prospective and recall collection.[89,93] This leaves the majority of studies primarily with a population comprised of male and female college students.

				Prospective Method				Recall Method		
	Author	Publication year	Country	Frequency	Format	Length captured	Responses submitted	Time between methods	Format	Length of recall
Vaginal	Allen	2007	Tanzania	Daily	SAQ (paper)	4 weeks	1 week	A few days	FTFI	4 week
	Durant	2000	USA	Daily	SAQ (paper)	2 months	1 week	Not mentioned	SAQ or FTFI	2 months
	Garry	2002	USA	Daily	eMail	4 weeks	Daily	6-12 months	Computer based survey	4 weeks
	Graham	2003	USA	Daily	SAQ (paper)	4 weeks	Daily	1, 2, or 3 months	SAQ (mailed)	4 weeks
	Hays	2001	USA	Daily	Single day page Weekly page Telephone	6 weeks	6 weeks	0-2 weeks	Not mentioned	6 weeks
	Hoppe	2008	USA	Daily	Mail Telephone	2 months	Daily	A few days	SAQ (mailed)	1 week 1 month 2 months
	Leigh	1998	USA	Daily	Telephone Weekly paper	4 weeks	Daily (phone) Weekly (paper)	A few days	SAQ (mailed)	4 weeks
	Minnis	2001	USA	Daily	SAQ (paper) Telephone	4 weeks	Daily for phone Weekly for paper	Immediately	Telephoned by interviewer	4 weeks
	Ramjee	1999	South Africa	Event based	SAQ (paper)	4 weeks	Monthly	Immediately	Not mentioned	2 week
	Tran	2013	Botswana	Weekly	SAQ (paper)	2 week	1 week	Immediately	SAQ (paper), group interview read aloud	2 week
Anal	Coxon	1999	UK	Daily	SAQ (paper)	4 week	4 week	Immediately	SAQ (mailed)	4 week
	Glick	2013	USA	Semi-weekly Once a week Bi-weekly	Web based	6 months	Varied	Up to 5 weeks	Web based	3 months
	Horvath	2007	USA	Daily	Web based	4 weeks	Daily	2 days	Web based	4 weeks
Both	McAuliffe	2007	USA	Daily	SAQ (paper)	3 months	Daily	up to 5 days	SAQ (paper) CASI ACASI	3 months

	Author	Sample size	Population gender and recruitment site, Ages Sexual orientation	Main finding Retrospective compared to prospective
Vaginal	Allen	150	Females at risk for HIV Heterosexual	Under-report sexual activity Significant difference for vaginal sex and male condom use
	Durant	120	Female college students Heterosexual	No significant difference between prospective and retrospective methods Over-report vaginal sex and condom use but not significant SAQs elicit more accurate results than FTFIs.
	Garry	37	Male and female college students 18-37 Heterosexual	Under-reported number of partners. Significant over-report number of sex acts vaginal, oral and anal sex and condom use
	Graham	75	Male and female college students 21-35 Heterosexual	Over-report # of vaginal sex in all monthly recall groups
	Hays	90	Females 18-35 Heterosexual	Kappa scores very good for number of sex acts between diaries, phone, and recall No directionality mentioned
	Hoppe	251	Male and female college students, patients at an STD clinic 14-35 Heterosexual	High consistency between methods. Most were consistent reporters.
	Leigh	79	Males and female college students and STI clinic patients 16-38	Overall no difference between daily and monthly Over-reported of vaginal sex in all monthly recall groups but not significant except in adolescents. No difference in condom reported use.
	Minnis	107	Females from Planned Parenthood 15-19 Heterosexual	Significant difference for number of sex acts and condom use contraceptive use was not reliable in retrospective, best was for hormonal at 0.71
	Ramjee	79	Female sex workers 18-44 Heterosexual	Under-reported sex acts and condom use
	Tran	161	Males in the defense force 18-30 Heterosexual	MEAN DIFFERENCE NOT SIGNIFICANT over report sex with regular noncohabiting partner underreport sex with spouse and sex for money
Anal	Coxon	74	Males MSM	Significant over-report of all sex acts Not significant for condom use
	Glick	95	Males 16–30 years MSM	No significant differences in the mean responses Under-reported for all measure. No difference in the diary schedules
	Horvath	45	Males >18 MSM	Most over reported, 28 and 85% of participants under- or over-reported Significant for receptive anal sex
Both	McAuliffe	493	Males and females MSM and heterosexuals	Under-reported anal or vaginal sex, and condom use Better when asked as by partner instead of aggregate; Better with casi; , MSM more consistent than heterosexuals

	Author	Difference for sex	Difference for condom use
Vaginal	Allen	Means: Prospective=13.01 Recall=7.91 p-value<0.001	Means: Prospective=3.65 Recall=1.96 p-value=0.002
	Durant	Discrepancy score z=0.21	Discrepancy score =2.07
	Garry	MANOVA F(1, 34) 17.86 p-value< .01	Wilcoxon's signed rank T(29) 60, p .02, f 0.34
	Graham	Estimated recall bias, ranged 0.9-2.5	Estimated recall bias, ranged 0.3-1.1
	Hays	Kappa >0.7	Kappa >0.6
	Hoppe	No difference	CONDOM USE 73% were consistent reporters, with 15% over-reporting and 12% underreporting
	Leigh	Mean difference (Recall-Prospective) = 0.66 r= 0.87	Mean difference (Recall-Prospective) = -0.02 r=0.96
	Minnis	Kappa=0.57	Kappa=0.34
	Ramjee	Mean difference 3.9 p-value<0.001	Mean difference 2.3 p-value<0.001
	Tran	Mean difference 0.48 p-value=0.29	Mean difference 2.37 p-value=0.12
Anal	Coxon	Relative difference (Recall-Prospective) /Recall -1.15 Receptive -1.00 Insertive	Relative difference (Recall-Prospective) /Recall -0.28 Receptive -0.52 Insertive
	Glick	Concordance correlation coefficient =0.84	Concordance correlation coefficient =0.88
	Horvath	Mean difference (Recall-Prospective): 1.6 Receptive 0.5 Insertive	Mean difference (Recall-Prospective): No condom use 0.5 Receptive 0.2 Insertive
Both	McAuliffe	Vaginal: Mean Prospective=34.59 Mean Recall=24.59 Anal: Means Prospective=18.99 Mean Recall=15.45	Vaginal: Mean Prospective=12.25 Mean Recall=9.82 Anal: Means Prospective=9.9 Mean Recall=6.91

Discussion

Overall, there is no consensus as to whether sexual behavior data from recall is similar to data collected via prospective methods. This is unfortunate given the relative convenience and cost effectiveness of using a single retrospective survey to collect information on sexual behavior.

This review was limited to articles published in English or articles that have been translated into English. There is the possibility of publication bias preventing the accessibility of data from studies that found no difference between different methods of sexual behavior data collection. There is no consistent "gold-standard" for method of collection; each study uses different methods to compare time spans and different collection techniques.

When examined separately, there appears to be a predilection for retrospectively over-reporting the number of anal sex acts amongst MSM. This may be due to recall errors or the desire to appear more sexually virile.[118,119,123] There was significant under-reporting amongst the populations that engaged in sex work. There was no significant difference in reporting for vaginal sex amongst heterosexuals, which did not involve sex work, FTFIs, or a long lag time between collection time points. Until further research is conducted, studies involving MSM or sexual behavior involving sex workers should conduct daily collection of sexual behavior.

In instances where there was a significant lag time, greater than 1 week, between the collection phases, there was a higher instance of data being over-reported. In order to prevent recall errors, studies will need to be designed such

that the collection of retrospective data occurs immediately, no more than 1 week after the time period of interest.

Due to the inherent sensitive nature of sexual behavior data it is advised that studies refrain from the use of FTFIs.[106] Although it may be appealing to have study personnel present to facilitate, it would be in the best interest of the data to design the survey such that it can be completed by the participant in private. Many articles included in this review used paper SAQ which is a viable option if the use of computers is prohibitive. The use of technology for data collection is ever growing and evolving; the method chosen to deliver the survey will have to consider the technical ability and access of the population.

Research in other fields has also found that recall was also comparable to prospective data collection. A 30-day recall of the volume of alcohol consumed was found to have high correlation (r=0.88) when compared to a daily diary collection.[129] The majority of articles included in this review had recall lengths of 1 month, limiting the applicability for studies that have longer periods of data collection. Further research will need to be conducted assessing the validity of recall lengths greater than 1 month. Accurate recall of greater than 1 month is possible; in a study on the adherence to oral contraceptives, researchers found substantial agreement between daily diaries and a 3-month recall.[130]

This review has several limitations. Each of the articles included conducted their studies using different methods that make a direct comparison difficult. This and the way in which they reported their results differed too greatly to be able to conduct a meta-analysis. Therefore there is some loss of detail by

having to report their results as an overall summation. Despite these limitations, this review provides a synopsis of recall comparative studies in the field of sexual behavior. Findings from this review may influence all areas of research where sexual behavior is involved, i.e. HIV/STI and reproductive studies.

In conclusion, there is compelling evidence that the use of a 1 month retrospective survey eliciting data on sexual behavior is equivalent to daily collection amongst heterosexuals, as long as the lag time between assessment and selected recall period is minimal.

CONCLUSION

TV is a very common STI that is often asymptomatic and is associated with a number of complications. Re-test positive, particularly among HIV+ women, is a frequent event. There are distinct advantages and disadvantages to each method of testing, whether it is increased sensitivity or time it takes for results. Choice of testing method(s) provide potentially different data for the study population. Optimal treatment regimens are still being determined by ongoing research and other potential co-infections need to be considered when examining reasons for a re-test positive.

ART and smoking are both independently associated with re-test positive TV infections which may result from possible treatment failure in HIV+ women. ART is potentially acting through the liver to interact with the metabolism of MTZ. Women enrolled in this study were on several different treatment regimens and the results demonstrate the ART effect is not limited to treatment regimens containing nevirapine. .At the TOC visit, women in the single dose MTZ arm were three times more likely to be TV positive if they were currently taking ART compared to those not on ART. While this interaction was seen in the single dose MTZ arm it may also occur in the multidose MTZ, however, the sample size was insufficient to detect the interaction.

Cigarette smoking causes changes in the vaginal environment and it is possible that smoking is interfered with the MTZ treatment causing the activity of the TV organism to be repressed to levels undetectable by culture methods. The

result from study 2 show that smoking a pack a day or more is associated with single dose MTZ re-test positive in HIV+ women at a 3 month follow-up visit. Since the association is only observed in the single dose arm it is possible that the longer length of treatment with multi-dose is able to overcome the effects of smoking on the vaginal flora and the women's innate immune response. These findings add to the evidence that abnormalities in the vaginal flora, like BV, increase the likelihood that a single dose of MTZ is inadequate in this patient population.

In the study assessing ART and the one on smoking, they both were able to account for the possibility of sexual re-exposure as a possible source for a positive TV test result at either the TOC visit or at the 3 month follow-up. The review of sexual behavior diaries provided evidence that a recall of sexual exposure after 1 month was similar to more frequent collection time points. The study on ART assessed sexual exposure history after 1 week; given the very short time period it is unlikely women did not accurately remember their sexual behaviors. However, the review paper was not able to assess recall periods greater than 1 month. So, the possibility remains that in the study on smoking, which used a single 3 month recall, women did not accurately remember their sexual exposures. More comparative research needs to be conducted prior to use of recall periods greater than 1 month in the proposed patient population.

This research into better co-exposure assessment and risk assessment methods, to avoid re-test positives, will help guide clinicians in choosing the most effective retesting and retreating options for their population.

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