The Influence of ART on the Treatment of *Trichomonas vaginalis* Among HIV-Infected Women

Alys Adamski,1 Rebecca A. Clark,2 Leandro Mena,2 Harold Henderson,2 Judy Levison,4 Norine Schmidt,1 Hirut T. Gebrekristos,1 David H. Martin,2 and Patricia Kissinger1

1Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana; 2Department of Medicine, Louisiana State University, New Orleans; 3Department of Medicine, University of Mississippi Medical Center, Jackson; and 4Baylor College of Medicine, Houston, Texas

**Objective.** Among women who are human immunodeficiency virus positive (HIV+), both prevalent and persistent infections with *Trichomonas vaginalis* (TV) are common. TV has been shown to increase vaginal shedding of HIV, which may influence HIV sexual and perinatal transmission, making prevention important. In 1 cohort of HIV+ women in Kenya, antiretroviral therapy (ART) use, mostly nevirapine based, was associated with lower cure rates of TV for single-dose therapy. Our goal was to repeat this study in a US-based cohort of HIV+/TV+ women and compare outcomes to those with multidose therapy.

**Methods.** A secondary data analysis was performed on a multicentered cohort of HIV+/TV+ women who were randomized to single-dose (2 grams) or 7-day (500 mg twice daily) multidose metronidazole (MTZ) treatment. Test of cure visit, via culture, occurred 6–12 days after treatment completion. Information was collected on sex partner treatment and sexual exposures. Persistent TV infection rates were compared for women on ART at baseline vs not on ART.

**Results.** Of the 226 women included, those on ART had more treatment failures than women not on ART (24/146 [16.4%] vs 5/80 [6.3%]; *P* = .03). When stratified by treatment arm, more treatment failures were seen in the single-dose arm (17/73 [23.3%] vs 3/39 [7.7%; ] *P* = .05) than in the multidose arm (7/73 [9.6%] vs 2/41 [4.8%]; *P* = .39).

**Conclusions.** ART usage was associated with a higher TV persistent infection rate among those receiving the single-dose treatment, but not the multidose, providing more evidence that multidose should be the preferred treatment for HIV+ women.

**Keywords.** antiretroviral; HIV; metronidazole; *Trichomonas vaginalis*.

Among women who are human immunodeficiency virus positive (HIV+), *Trichomonas vaginalis* (TV) is the most common curable sexually transmitted infection [1]. It has been associated with increased genital shedding of HIV, pelvic inflammatory disease, and adverse pregnancy outcomes [2–8]. Successful treatment of TV has been shown to reduce the genital shedding of HIV, and this may be an effective strategy for reducing the incidence of HIV transmission [9,10]. While TV is generally susceptible to metronidazole (MTZ) [11,12], high rates of persistent TV infections among HIV+ women have been reported (18%–36%), suggesting that clinical treatment failure is an issue [2,13–16]. There is growing evidence that single-dose therapy is inadequate for preventing clinical treatment failure of TV in HIV+ women [2,11–14].

Our previous study found that the multidose (500 mg MTZ twice daily for 7 days) was superior to the single dose (2 grams MTZ) in preventing clinical treatment failure among HIV+ women [15]. In a secondary analysis of these data, it was found that bacterial vaginosis (BV) interferes with the single-dose treatment, leading to higher
clinical failure rates in women coinfected with BV [17]. In contrast, women in the multidose arm had the same clinical failure rate regardless of their BV status.

In a recent study of HIV+/TV+ women in Kenya, Balkus et al. presented evidence that antiretroviral therapy (ART), mostly neviripine based, interacts with MTZ treatment [18]. They found that a 2-gram single dose of MTZ had a lower success rate in women who were on ART compared with those not on ART. Our purpose in this study was to determine if clinical treatment failure is amplified in women on ART in a US-based cohort with a wider variety of ART and to determine if this pattern is also found in women receiving multidose treatment.

**METHODS**

**Study Population**

Data were collected during a previously reported randomized control trial (RCT) for the treatment of TV among HIV+ women [15]. HIV+ women attending public HIV outpatient clinics in New Orleans, Louisiana; Houston, Texas; and 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), aged ≥18 years, English speaking, TV positive by culture, willing to take MTZ, and able 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 or breastfeeding, incarcerated, taking disulfiram, or had been treated with MTZ within the previous 14 days.

At enrollment, women completed an audio computer-assisted self-interview to assess their demographic information, current medications including ART, and other factors that have been found to be associated with persistent TV infections, such as smoking [19], alcohol use [20], vaginal douching practices [21], and vaginal sexual history for the last 3 months. 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. Using a chart with pictures of medications available during the study period (May 2006–July 2009), a trained study staff person asked which HIV medications were used. When ART status was discrepant between medical records and patient-report, the patient-report was used. ART adherence was assessed by asking the woman, “did you take this medication as prescribed the day before?” BV was diagnosed in women who had a Nugent score ≥7. CD4 cell count and plasma viral load were abstracted from the women’s clinic charts to determine the most recent results in the 3 months prior to the baseline visit.

Eligible women who agreed to participate in the study were randomized to receive either 2 grams MTZ (single dose) or 500 mg MTZ twice daily for 7 days (multidose). Women in both treatment arms were given 2 grams single-dose MTZ to deliver to their sexual partner(s), up to 4 partners. In prior analyses, women reported high rates of partner treatment (75%) [22] and high rates of treatment completion (97%) [22].

A test-of-cure (TOC) visit was conducted 6–12 days post treatment completion [23,24]. Women were tested for the presence of TV by culture and completed a survey assessing possible sexual reexposures and treatment compliance. Sexual reexposure was defined as having unprotected sex with a baseline partner prior to completion of medication by participant and/or partner.

**Laboratory Procedures**

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 (Biomed Diagnostics, White City, OR) per packet insert instructions. Vaginal swabs were either self- or provider-collected and used to inoculate the InPouch culture per the manufacturer’s protocol. The presence of BV was determined by collecting a vaginal swab that was rolled onto a slide and air dried. The slide was later sent to a central laboratory for gram staining and Nugent scoring [25].

**Statistical Analysis**

Statistical analysis was conducted using SAS 9.2. HIV+ women who reported ART usage at baseline were compared with women not using ART. Covariates collected at baseline to be assessed for possible confounding included treatment arm, BV status, CD4 cell count, plasma viral load levels, and sexual reexposure status. 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. The outcome of interest was a positive TV culture at TOC. Relative risk with a 95% confidence interval was calculated by χ² test to assess the relationship between ART usage and persistent TV infection at TOC.

The original RCT was 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. The Tulane University Institutional Review Board approved this secondary analysis as exempted research.

**RESULTS**

Of the 270 HIV+ and TV+ women enrolled in the RCT, 226 attended their TOC visit and had complete data for analysis. Table 1 presents demographic information for enrolled women. The majority of women were black (92%) and unemployed (70%). More than half of the women (65%, n = 146) were on ART at their baseline visit. Of those on ART, 93.8% reported...
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 of the women reporting being a regular smoker (44%) and more than a third reported drinking alcohol in the past week (38%). A majority of the women had BV (67%) as diagnosed by Nugent score of ≥7. Similarly, 67% of women reported that they had douched in the last 30 days.

In the 3 months prior to enrollment, 76% of women reported having 1 or more sexual partners (either male or female). Demographic information did not differ based on whether the women were on ART or not on ART at baseline. Women who were on ART at baseline were more likely to have CD4 cell counts <200/mm³ (23% vs 17%; \( P = .003 \)). A CD4 cell count ≤200/mm³ was associated with a positive TOC only in those women who were receiving the multidose treatment. Viral load was not associated with a positive TOC in either treatment arm. Viral loads >10 000 copies/mm were common in women not on ART (50%) when compared with those on ART (27%; \( P = .0007 \)).

The distribution of types of ART taken by women at baseline is shown in Table 2. Of women currently on ART, nearly all were on a nucleoside reverse transcriptase inhibitor (NRTI) (95%), a majority were on a protease inhibitor (61%), with a few on a nonnucleoside 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% confidence interval, 1.04–6.63) for women on ART compared with those not on ART (\( P = .03 \)). When examined by treatment arm and ART usage, the association was found only in the single-dose arm (\( P = .05 \)) and not in the multidose arm (\( P = .39 \); Table 3).

BV was equally distributed between the 2 groups of women on ART (67.8%) and not on ART (65.0%; \( P = .67 \)) overall and was equally distributed when examined by treatment arm. A plasma viral load >10 000 was associated with the women being prescribed ART (\( P = .0007 \)) but was not independently associated with the woman experiencing treatment failure (\( P = .600 \)). When stratified by treatment arm, plasma viral load remained unassociated with treatment failure, single dose (\( P = .180 \)) and 7-day dose (\( P = .106 \)). CD4 cell count was not associated with treatment failure overall (\( P = .295 \)). However, when stratified by treatment arm, it was significant for the 7-day dose (\( P = .033 \)) and not the single dose (\( P = 1.00 \)).

### Table 1. Baseline Characteristics of Human Immunodeficiency Virus–Positive/Trichomonas vaginalis–Positive Women (N = 226) by Antiretroviral Therapy Status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ART Yes n = 146 (%)</th>
<th>ART No n = 80 (%)</th>
<th>( P ) Value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>133 (91.1)</td>
<td>74 (92.5)</td>
<td>.74</td>
</tr>
<tr>
<td>Unemployed</td>
<td>108 (73.9)</td>
<td>50 (62.5)</td>
<td>.10</td>
</tr>
<tr>
<td>Did not graduate high school</td>
<td>59 (40.4)</td>
<td>32 (40.0)</td>
<td>.95</td>
</tr>
<tr>
<td>Married or cohabitating</td>
<td>34 (23.3)</td>
<td>25 (31.3)</td>
<td>.19</td>
</tr>
<tr>
<td>Regularly smokes cigarettes</td>
<td>61 (41.8)</td>
<td>37 (46.2)</td>
<td>.52</td>
</tr>
<tr>
<td>Drank alcohol in the past week</td>
<td>52 (35.6)</td>
<td>34 (42.5)</td>
<td>.31</td>
</tr>
<tr>
<td>Has vaginally douch in the past 30 days</td>
<td>101 (69.2)</td>
<td>52 (65.0)</td>
<td>.52</td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>99 (67.8)</td>
<td>52 (65.0)</td>
<td>.67</td>
</tr>
<tr>
<td>≥1 sex partner in past 3 months</td>
<td>106 (72.6)</td>
<td>67 (83.7)</td>
<td>.06</td>
</tr>
<tr>
<td>Multiday dose metronidazole(^b)</td>
<td>73 (50.0)</td>
<td>41 (51.3)</td>
<td>.86</td>
</tr>
<tr>
<td>Sexual reexposure</td>
<td>2 (1.3)</td>
<td>3 (3.7)</td>
<td>.57</td>
</tr>
<tr>
<td>CD4 cell count ≤200/mm³</td>
<td>53 (23.4)</td>
<td>14 (17.5)</td>
<td>.003</td>
</tr>
<tr>
<td>Viral load &gt;10 000 copies</td>
<td>40 (27.4)</td>
<td>40 (50)</td>
<td>.0007</td>
</tr>
</tbody>
</table>

Abbreviation: ART, antiretroviral therapy.

\(^a\) \( P \) values obtained by \( \chi^2 \) analysis.

\(^b\) Patient randomized to receive the multiday dose of 500 mg twice daily for 7 days vs the single dose of 2 grams metronidazole.

### Table 2. Types of Antiretroviral Therapy (ART) Prescribed Among Human Immunodeficiency Virus–Positive Women Currently Receiving any ART

<table>
<thead>
<tr>
<th>Antiretroviral Therapy Class</th>
<th>n = 146 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleoside reverse transcriptase inhibitor</td>
<td>138 (95)</td>
</tr>
<tr>
<td>Protease inhibitor</td>
<td>89 (61)</td>
</tr>
<tr>
<td>Nonnucleoside reverse transcriptase inhibitor</td>
<td>53 (36)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (3)</td>
</tr>
</tbody>
</table>

### Table 3. Post Treatment Trichomonas vaginalis Infection Rates at Test-of-Cure Visit by Antiretroviral Therapy Status and Metronidazole Treatment Arm Among Human Immunodeficiency Virus–Positive Women (N = 226)

<table>
<thead>
<tr>
<th></th>
<th>Percentage Overall Persistent Infection Rate TV+ (n)</th>
<th>Percentage Persistent Infection Rate TV+ on ART</th>
<th>Percentage Persistent Infection Rate TV+ Not on ART</th>
<th>Relative Risk (95% confidence interval)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test of cure</td>
<td>12.8 (29/226)</td>
<td>16.4 (24/146)</td>
<td>6.3 (5/80)</td>
<td>2.63 (1.04–6.63)</td>
<td>.036</td>
</tr>
<tr>
<td>Single dose</td>
<td>17.9 (20/112)</td>
<td>23.3 (17/73)</td>
<td>7.7 (3/39)</td>
<td>3.16 (.99–10.08)</td>
<td>.052</td>
</tr>
<tr>
<td>Multiday dose</td>
<td>7.9 (9/114)</td>
<td>9.6 (7/73)</td>
<td>4.8 (2/41)</td>
<td>1.97 (.43–9.03)</td>
<td>.385</td>
</tr>
</tbody>
</table>

Abbreviations: ART, antiretroviral therapy; TV, Trichomonas vaginalis
However, when included in an adjusted model, it did not significantly change the effect estimate and was thus excluded.

Another analysis was conducted to determine if the association was still present in treatment regimes that did not contain an NNRTI. Given that only 36% of women were currently prescribed an 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 reexposure was not included in the final model for analysis, as none of the women who were TV+ at TOC met the criteria for sexual reexposure and it was equally distributed between ART statuses. Of 226 women seen for their TOC 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 (unwashed sex toys or mutual masturbation) with baseline female partners.

**DISCUSSION**

Both the parent study and the secondary data analysis of the interaction between BV and treatment found that in HIV+ women the multidose 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. Their longer follow-up and study population, female sex workers, may explain why their cohort had 50% sexual exposure compared with 2% in our cohort. HIV+ women on ART in the Balkus cohort were prescribed nevirapine-containing regimens, whereas in our cohort the vast majority of women on an NNRTI were prescribed efavirenz. Only one woman received nevirapine. Our results demonstrate that 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 that we achieved only 60% power for the whole cohort, 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. Although InPouch is more sensitive than wet mount microscopy (which was used in the Balkus et al study), it is not as sensitive as NAAT. It is possible that attenuated TV infections were missed at TOC. However, performing a nucleic test for TOC prior to 3 weeks post treatment completion is not recommended as there may be remnant TV DNA present, and the nucleic test will be falsely positive for an active infection, thus we decided that culture was the best testing approach.

This study cannot 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. To further elucidate the interaction between ART and MTZ, it is recommended 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-gram MTZ dose and may occur in the multidose MTZ; however, the sample size was insufficient to detect the interaction. In conclusion, our data show that multidose MTZ may be superior to single-dose MTZ in the treatment of TV among HIV+ women on ART.

**Notes**

**Acknowledgments.** We thank the patients and staff from the participating clinics: HIV Outpatient Clinic and NOAIDS in New Orleans, Louisiana; Crossroads Clinic in Jackson, Mississippi; and the Thomas Street and Northwest Clinics in Houston, Texas.

**Disclaimer.** This article's content is solely the responsibility of the authors and do not necessarily represent the official views of National Institutes of Health (NIH).

**Financial support.** The project described was supported by the NIH - National Institute of Allergy and Infectious Disease grant number U19 260 A1061972.

**Potential conflicts of interest.** All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


