Correlation of Leukorrhea and *Trichomonas vaginalis* Infection

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*Trichomonas vaginalis* is a common sexually transmitted infection (STI) causing vaginitis. Microscopy has poor sensitivity but is used for diagnosis of trichomoniiasis in resource-poor settings. We aimed to provide a more reliable diagnosis of trichomoniiasis by investigating an association with leukorrhea. Women presenting for evaluation of vaginal discharge, STI exposure, or preventative gynecologic examination were evaluated for *Trichomonas* infection. Vaginal pH was determined and microscopy was performed by the provider, who recorded the number of polymorphonuclear leukocytes (PMNLs) per epithelial cell and the presence of clue cells, yeast, and/or motile trichomonads. Leukorrhea was defined as greater than one PMNL per epithelial cell.

Culture and a nucleic acid amplification test (NAAT) were used to detect *T. vaginalis*. Patients were evaluated for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using NAATs and bacterial vaginosis using Gram stains. Two hundred ninety-four women were enrolled, and 16% were found to have *Trichomonas* (46/294). *Trichomonas* infection was more common in parous non-Hispanic, black women, who reported low rates of contraceptive use (33% versus 17%; *P* = 0.02) and a STI history (85% versus 55%; *P* = 0.002). These women were more likely to report vaginal discharge (76% versus 59%; *P* = 0.02) and have an elevated vaginal pH (87% versus 48%; *P* < 0.001) and gonorrhea infection (15% versus 4%; *P* = 0.002). Leukorrhea was associated with a 4-fold-increased risk of *Trichomonas* infection. Leukorrhea on microscopy was associated with *Trichomonas* vaginitis. Patients with leukorrhea should be evaluated with more-sensitive tests for *T. vaginalis*, preferably NAATs, if microscopy is negative.

The prevalence of trichomoniiasis in U.S. women is more than the combined prevalences of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections (1). Infection with *Trichomonas* is associated with a significant risk of morbidity in women, including pelvic inflammatory disease, adverse pregnancy outcomes (e.g., preterm labor and delivery), cervical dysplasia, infertility, increased risk of postoperative infection, and HIV acquisition and transmission (2–5). U.S. health care costs related to trichomoniiasis approach 34 million dollars per year (1).

*Trichomonas vaginalis* is a parasitic flagellate that adheres to and engulfs vaginal epithelial cells through phagocytosis. The resulting death of epithelial cells leads to inflammation within the genital tract and an increased number of polymorphonuclear leukocytes (PMNLs) in vaginal fluid (6). An increase in PMNLs noted on microscopy of vaginal secretions indicates an infectious or inflammatory process and may be a useful screening tool when evaluating vaginal discharge.

Leukorrhea refers to the presence of PMNLs in vaginal fluid, which can be quantified as the number of PMNLs seen on microscopy. Previous studies have defined leukorrhea based on the number of PMNLs per high-power field (hpf). Leukorrhea is considered present if there are more than one PMNL per oil immersion field (×1,000) or more than 10 PMNLs per hpf (×400) (7, 8). Leukorrhea has also been described according to the ratio of PMNLs to vaginal epithelial cells; more than 1 PMNL per epithelial cell is consistent with leukorrhea (9).

Noninfectious conditions, such as pregnancy, menstruation, or infertility, are associated with an increased number of PMNLs in vaginal fluid (10–12). Genital tract infections, such as *C. trachomatis* cervicitis and endometritis, are strongly associated with leukorrhea (8). This association of leukorrhea and *C. trachomatis* infection is true in pregnant and nonpregnant women. Leukorrhea is a strong predictor for *C. trachomatis* infection in nonpregnant and pregnant women (relative risk [RR], 59; 95% confidence interval [CI], 8 to 413; versus RR, 16; 95% CI, 7 to 32) (7). The absence of leukorrhea has a high negative predictive value (95%) for the diagnosis of upper genital tract infection, i.e., endometritis (8).

There are currently no studies in the English literature for which a correlation between leukorrhea in vaginal secretions and *Trichomonas* infection is reported. This information would be clinically useful when evaluating vaginal discharge and lower genital tract symptoms. Microscopy is the most common method used to diagnose trichomoniiasis, but the sensitivity is poor (55%; 35 to 77%) (13–16). The presence of leukorrhea in the absence of motile trichomonads could potentially be presumed to be cervicitis and empirically treated without further evaluation for *Trichomonas*. A known significant relationship between leukorrhea on microscopy and *Trichomonas* might prompt clinicians to screen women in this clinical scenario for this common infection using more-sensitive methods.

The objective of this study was to determine an association between the presence of leukorrhea on microscopy of vaginal secretions and *Trichomonas* infection.

**MATERIALS AND METHODS**

The study was approved by the Medical University of South Carolina Institutional Review Board (HR no. 19180). Two hundred ninety-four...
two hundred ninety-four women, ages 16 to 67, were enrolled in this study. More than 60 percent of the subjects presented for evaluation of a vaginal discharge (179). The majority of subjects were non-Hispanic, black (216) and currently unmarried (260). Ninety-eight percent of women reported being exclusively heterosexual (287), and more than half had a history of an STI (175). Most women reported using a method of contraception, including condoms (234). Other patient characteristics are listed in Table 1.

Trichomonas testing results were available for almost all 294 women enrolled in the study: 292 T. vaginalis cultures and 247 nucleic acid amplification tests. Sixteen percent (46) of the study subjects had a positive Trichomonas test result. Women with a Trichomonas infection were more likely to be parous (88% versus 64%; P = 0.002) and non-Hispanic black (93% versus 70%; P = 0.0004). They were less likely to use any form of contraception (33% versus 17%; P = 0.02), but rates of condom use were similar in the two groups (4% versus 8%; P = 0.5). Women with Trichomonas infection were more likely to report a history of STIs (85% versus 55%; P = 0.002) (Table 2).

According to Nugent’s criteria, 41% of women in the study had BV (119/289). Five Gram stains could not be scored due to inadequate specimen collection. The rates of BV determined by Nugent’s criteria and high-power microscopy findings of clue cells were equal (both 41%), but the agreement between these tests was low (kappa statistic = 0.5). The rates of BV were similar among the women infected with Trichomonas versus those not infected (43% versus 41%; P = 0.7). PMNLs were noted on 71% of all Gram stain specimens. Compared to uninfected women, women with T. vaginalis were more likely to have PMNLs on Gram stain.

### RESULTS

#### Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr) (mean ± SD)</td>
<td>28 ± 9</td>
</tr>
<tr>
<td>Parity, median (interquartile range)</td>
<td>1.4 (0–8)</td>
</tr>
<tr>
<td>% of patients (no. of patients/total) with characteristic</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic, black race</td>
<td>74 (216/293)</td>
</tr>
<tr>
<td>Hispanic race</td>
<td>0.6 (2/293)</td>
</tr>
<tr>
<td>Single marital status</td>
<td>90 (260/290)</td>
</tr>
<tr>
<td>Exclusively heterosexual partner preference</td>
<td>98 (287/292)</td>
</tr>
<tr>
<td>Vaginal douching</td>
<td>16 (46/286)</td>
</tr>
<tr>
<td>Regular menstrual cycles</td>
<td>63 (170/272)</td>
</tr>
<tr>
<td>Currently not using contraception (including condoms)</td>
<td>20 (58/292)</td>
</tr>
<tr>
<td>Report condoms only for contraception</td>
<td>7 (22/294)</td>
</tr>
<tr>
<td>Hormonal contraception</td>
<td>58 (169/292)</td>
</tr>
<tr>
<td>Surgical contraception</td>
<td>14 (41/292)</td>
</tr>
<tr>
<td>History of abnormal cervical cytology</td>
<td>17 (47/275)</td>
</tr>
<tr>
<td>Multiple sexual partners</td>
<td>24 (68/278)</td>
</tr>
<tr>
<td>Patient reported vaginal discharge</td>
<td>61 (179/292)</td>
</tr>
<tr>
<td>History of one prior sexually transmitted infection (STI)</td>
<td>60 (175/292)</td>
</tr>
<tr>
<td>History of more than one STI</td>
<td>49 (84/170)</td>
</tr>
<tr>
<td>History of Trichomonas infection</td>
<td>40 (66/170)</td>
</tr>
<tr>
<td>History of Chlamydia infection</td>
<td>62 (106/170)</td>
</tr>
<tr>
<td>History of gonorrhea infection</td>
<td>37 (61/166)</td>
</tr>
<tr>
<td>History of genital herpes</td>
<td>12 (21/170)</td>
</tr>
</tbody>
</table>

*Some patient characteristic data were not available for all participants.

Regular menstrual cycles defined as monthly menses every 21 to 35 days.

Surgical contraception may include female tubal ligation or male vasectomy.

Defined as >1 partner in the 12 months preceding evaluation.

#### Statistical analyses

Continuous variables (age and parity) were analyzed using Student’s t test to compare means and the Wilcoxon rank sum test to compare medians. For dichotomous variables, chi-square and Fisher’s exact tests were used to compare women with and without Trichomonas infection. Uni- and multivariate logistic regression analyses were used to determine factors associated with Trichomonas infection. Variables with a P value of ≤0.2 were included in multivariate logistic regression analyses. Kappa statistics were calculated to determine the agreement between Trichomonas culture and NAATs. Chi-square tests were used to calculate the sensitivity, specificity, and positive and negative predictive values of culture, visualization of motile trichomonads, and leukorrhea on microscopy for the diagnosis of trichomoniasis.
Determination of *Trichomonas vaginalis* infection and those not infected

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value for group*</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr), mean (± SD)</td>
<td>Trichomonas infection: 29.3 (±10.8)</td>
<td>No Trichomonas: 27.7 (±8.6)</td>
</tr>
<tr>
<td>Parity, median (interquartile range)</td>
<td>2 (0–6)</td>
<td>1 (0–8)</td>
</tr>
</tbody>
</table>

% with characteristic (no. of women/total)

- Non-Hispanic, black race: 93 (43/46) vs. 70 (173/247); 0.0004
- Single marital status: 91 (41/45) vs. 89 (219/245); 0.7
- Exclusively heterosexual: 98 (45/46) vs. 98 (242/246); 0.8
- Douching: 20 (9/45) vs. 15 (37/241); 0.4
- Regular menstrual cycles: 49 (19/39) vs. 65 (151/233); 0.06
- No contraception, including condoms: 33 (15/46) vs. 17 (43/246); 0.02
- Reported condom use: 4 (2/46) vs. 8 (20/248); 0.5*
- History of abnormal cervical cytology: 15 (7/46) vs. 17 (40/229); 0.7
- History of at least one STI: 85 (39/46) vs. 55 (136/246); 0.002
- Reported vaginal discharge: 76 (35/46) vs. 59 (144/246); 0.02
- Abnormal vaginal pH*: 87 (40/46) vs. 48 (120/248); <0.0001
- PMNLs on microscopy: 78 (36/46) vs. 49 (118/240); 0.0003
- PMNLs on Gram staining: 87 (40/46) vs. 69 (167/243); 0.01
- Bacterial vaginosis: 43 (20/46) vs. 41 (99/243); 0.7
- *Chlamydia* infection: 7 (3/46) vs. 4 (11/248); 0.5*
- Gonorrhea infection: 15 (7/46) vs. 4 (9/248); 0.002

*For the *Trichomonas*-infected group, n = 46; for the uninfected group, n = 253.

*Abnormal vaginal pH is defined as a pH of >4.5.

*Bacterial vaginosis is defined as a Nugent score of ≥7 (17).

* Determination of *Chlamydia* and gonorrhea infection was based on positive NAATs.

*+, result of Fisher’s exact test for small sample size. Otherwise, P values were calculated using chi-square tests.

The visualization of any PMNLs on Gram staining (hpf, ×1,000) was a more-sensitive test for *Trichomonas* infection than leukorrhea on microscopy (hpf, ×1,000; 90% versus 78%). However, the specificity of Gram staining was lower than that of leukorrhea on microscopy. The PPV for leukorrhea assessed by Gram staining versus the PPV for assessment by microscopy were similar, 21 and 25%, respectively (Table 4).

**DISCUSSION**

The incidence of *Trichomonas vaginalis* infection among the women in this study population (16%) is five times higher than...
with Trichomonas infection in order to identify patients at risk and use tests more sensitive than microscopy, such as culture or NAATs, to diagnose Trichomonas infection.

Similar to studies of other genital tract infections, leukorrhea in vaginal secretions was associated with Trichomonas infection. This is physiologically plausible, because Trichomonas causes increased inflammation within the vaginal environment and has been found to “attract” PMNLs (6). Specifically, CD4 lymphocytes are recruited to the site of Trichomonas infection (20).

In the absence of leukorrhea on microscopy (>1 PMNL per epithelial cell), Trichomonas infection was unlikely (OR, 0.3; 95% CI, 0.1 to 0.8). Although gonorrhea cervicitis was associated with an increased risk of Trichomonas infection, this did not impact the association between leukorrhea and Trichomonas vaginitis. When controlling for the presence of gonorrhea, Chlamydia trachomatis infection, and bacterial vaginosis, patients were 4 times more likely to have Trichomonas if leukorrhea was noted on microscopy (OR, 3.6; 95% CI, 1.6 to 7.6). Our findings suggest that the presence of leukorrhea on microscopy should increase clinicians’ suspicion of Trichomonas infection and lead to further evaluation with more-sensitive tests if microscopy is negative for motile trichomonads.

The Aptima T. vaginalis analyte-specific reagent used in this study has emerged as the most sensitive test for detection of Trichomonas infections (4, 21–23). Because concomitant C. trachomatis and N. gonorrhoeae infections are common in patients with trichomoniases, a panel of NAATs for these three infectious agents would be ideal for comprehensive STI diagnosis. The Aptima T. vaginalis assay was recently cleared by the FDA for diagnostic use. This should greatly increase availability of the test, with cost and turnaround time for results similar to those of the Aptima C. trachomatis and N. gonorrhoeae assay.

The association between leukorrhea and Trichomonas vaginitis in our population is both physiologically plausible and likely applicable to other populations seeking evaluation for vaginal discharge or gynecologic exam. Our study subjects are not representative of a general population given the young age of participants, high rates of reported and concurrent STIs, and disproportionate representation of minorities. However, this population is similar to others studied in urban centers, STI clinics, and family planning centers. It is in these low-resource settings that microscopy is often used for the diagnosis of Trichomonas. Our findings suggest that the presence of leukorrhea on microscopy should increase clinical suspicion for Trichomonas vaginitis, prompting additional tests, and may enhance identification of this common and morbid infection.

### Table 4

<table>
<thead>
<tr>
<th>Method or target</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
<th>% PPV</th>
<th>% NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>93 (38/41)</td>
<td>100 (205/205)</td>
<td>100 (38/38)</td>
<td>99 (205/208)</td>
</tr>
<tr>
<td>Motile trichomonads</td>
<td>51 (21/41)</td>
<td>98 (200/204)</td>
<td>84 (21/25)</td>
<td>91 (200/220)</td>
</tr>
<tr>
<td>Leukorrhea on microscopy</td>
<td>78 (32/41)</td>
<td>51 (102/199)</td>
<td>25 (32/129)</td>
<td>92 (102/111)</td>
</tr>
<tr>
<td>Motile trichomonads or leukorrhea</td>
<td>78 (32/41)</td>
<td>53 (109/207)</td>
<td>25 (32/130)</td>
<td>92 (109/118)</td>
</tr>
<tr>
<td>PMNLs on Gram staining</td>
<td>90 (37/41)</td>
<td>33 (68/207)</td>
<td>21 (37/176)</td>
<td>94 (68/72)</td>
</tr>
</tbody>
</table>

*Parenthetical numbers are no. of correct results/no. tested. PPV, positive predictive value; NPV, negative predictive value.*
ACKNOWLEDGMENTS

We acknowledge Gen-Probe, Inc., for their support and funding of this research.

We thank the staff of the MUSC Microbiology and Molecular Pathology labs for their tireless work in analyzing the additional specimens required for this study. We especially recognize April Kegl.

The coinvestigator Fredrick Nolte has been a member of the Gen-Probe Scientific Advisory Board from 2007 to the present. The annual compensation associated with this activity is approximately $10,000. Gen-Probe products were used to determine the Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis infection status of the patients described in our article.

REFERENCES