Clinical Evidence for the Role of *Trichomonas vaginalis* in Regulation of Secretory Leukocyte Protease Inhibitor in the Female Genital Tract

Jill S. Huppert,1 Bin Huang,2 Chen Chen,2 Hassan Y. Dawood,3 and Raina N. Fichorova3

1Division of Gynecology and 2Division of Epidemiology and Biostatistics, Cincinnati Children’s Hospital Medical Center, Ohio; and 3Laboratory of Genital Tract Biology, Department of Obstetrics, Gynecology and Reproductive Biology, Harvard Medical School and Brigham and Women’s Hospital, Boston, Massachusetts

**Background.** Secretory leukocyte protease inhibitor (SLPI) is responsible for regulating inflammatory damage to and innate and adaptive immune responses in the vaginal mucosa. Depressed cervicovaginal SLPI levels have been correlated with both *Trichomonas vaginalis* infection and poor reproductive health outcomes.

**Methods.** We measured levels of SLPI in 215 vaginal specimens collected from adolescent and young adult females aged 14–22 years. Log-transformed SLPI values were compared by analysis of variance or by an unpaired t test before and after adjustment for confounding effects through the propensity score method.

**Results.** Females receiving hormonal contraceptives and those with an abnormal vaginal pH had lower SLPI levels as compared to their peers. After propensity score adjustment for race, behavioral factors, hormonal use, and other sexually transmitted infections (STIs), SLPI levels were lower in females with a positive *T. vaginalis* antigen test result, a vaginal pH >4.5, vaginal leukocytosis, and recurrent (vs initial) *T. vaginalis* infection, with the lowest levels observed in those with the highest *T. vaginalis* loads.

**Conclusions.** The SLPI level was reduced by >50% in a *T. vaginalis* load–dependent manner. Future research should consider whether identifying and treating females with low levels of *T. vaginalis* infection (before they become wet mount positive) would prevent the loss of SLPI and impaired vaginal immunity. The SLPI level could be used as a vaginal-health marker to evaluate interventions and vaginal products.

**Keywords.** SLPI protein; human; *Trichomonas vaginalis*; Vaginosis; Bacterial; Sexually Transmitted Diseases; Adolescent.
antibiotic treatment of *T. vaginalis* does not alter the risk [11, 12], suggesting that immunologic changes triggered by *T. vaginalis* infection may persist after parasitological cure. *T. vaginalis* is known to cause vaginal disruption, as evidenced by an elevated vaginal pH, vaginal leukocytosis, and altered vaginal mucosal immune response [4, 9, 13, 14]. Elevated vaginal pH is associated with sexually transmitted infections (STIs), bacterial vaginosis [15], and poor pregnancy outcomes [16]. Vaginal leukocytosis is associated with endometritis [17]. The SLPI level is lower in women infected with *T. vaginalis* as compared to women with *Chlamydia trachomatis* infection, *Neisseria gonorrhea* infection, bacterial vaginosis, or no infection [18]. In vitro, SLPI is digested by *T. vaginalis* cysteine proteases [19].

At present, there are few studies of SLPI that include large numbers of young nonpregnant females, many of whom use hormonal contraception, which, on its own, has controversial effects on the mucosal immune function in adolescents [20, 21]. In addition, *T. vaginalis* infections vary from asymptomatic infections detected by nucleic amplification testing only to infections associated with severe vaginal symptoms and a positive result of wet mount testing. Although no lasting acquired immunity has been associated with *T. vaginalis* [9], initial *T. vaginalis* infections may behave differently than recurrent or persistent infections in term of innate immune environment. We sought to determine whether vaginal levels of SLPI correlated with *T. vaginalis* loads, initial versus recurrent *T. vaginalis* infections, hormonal contraceptive use, and traditional measures of vaginal disruption.

**METHODS**

**Clinical Study Design**

This study was part of a larger cross-sectional study assessing the accuracy and acceptability of self-testing for *T. vaginalis*, the results of which are detailed in our previous work [22]. This analysis and the original study were approved by the hospital’s institutional review board. Briefly, adolescent and young adult females ages 14–22 years were recruited from an adolescent clinic and the emergency department. A clinician performed a pelvic examination and collected multiple vaginal swab specimens for immediate testing. One polyester swab was frozen dry at −80°C and stored for future use and testing. Participants completed questionnaires encompassing demographic characteristics, sexual history (including prior STI history, number of sex partners, and use of contraceptives), and vaginal or gynecological symptoms.

Clinical bacterial vaginosis was defined using modified version of the Amsel criteria (ie, an abnormal vaginal pH [>4.5], a high clue cell percentage [>20% of epithelial cells per field], and a positive result of an amine test). Compared with Nugent Gram staining, these objective criteria provide sensitivity and specificity similar to that of the full Amsel criteria [23, 24]. Hormonal contraception in the prior 3 months was characterized as none; combined estrogen and progestin (pills, ring, or patch); or progestin only (depot medroxyprogesterone acetate or progestin-containing intrauterine device).

**Laboratory Methods**

As described previously [25], vaginal swab specimens were used for wet mount diagnostic testing and for assessment of pH, amines, sialidase, and STIs. Leukocytosis on wet mount testing (magnified at 400×) was categorized as low (≤5 leukocytes per field) or high (≥6 leukocytes per field) [26]. Clue cells (vaginal epithelial cells with adherent bacteria) were categorized as present (>20% of epithelial cells per field) or absent [27]. Vaginal pH (pHydrion, Mikro Essentials Laboratories, Brooklyn, NY) was classified as >4.5 (elevated) or ≤4.5 (normal) [28]. Amines were recorded if a fishy odor was detected after applying potassium hydroxide to the specimen [27]. The wet mount test was deemed positive for *T. vaginalis* if motile trichomonads were observed on direct microscopy. Sialidase testing (BVBlue, Sekisui Diagnostics, Cambridge, MA), the rapid antigen *T. vaginalis* test (OSOM *T. vaginalis* Trichomonads Rapid Test, Sekisui Diagnostics), and *T. vaginalis* culture (InPouch *T. vaginalis* culture, BioMed, White City, OR) were performed following manufacturers’ directions. On the basis of prior work, we considered any positive *T. vaginalis* test to be a true positive *T. vaginalis* infection [29].

At study closure, the stored vaginal swab sample was brought to room temperature and eluted with 1.0 mL of phosphate-buffered saline for 10 minutes. One half of the eluent was transferred to a vaginal swab collection kit (Aptima, Gen-Probe, San Diego, CA) and transported on ice to the International Sexually Transmitted Diseases Research Laboratory (Baltimore, MD). *C. trachomatis* and *N. gonorrhea* were detected using nucleic acid amplification testing (NAAT; Aptima Combo2, Gen-Probe). *T. vaginalis* and *Mycoplasma genitalium* were detected on the same NAAT platform, using research-use-only reagents.

The remaining eluent (0.5 mL) was transferred to a sterile vial, stored frozen at −80°C, and shipped frozen to the Laboratory of Genital Tract Biology at Brigham and Women’s Hospital, where samples were assayed for total protein content and SLPI. The use of these samples for biomarker analysis was approved by the Brigham and Women’s Institutional Review Board for Human Subject Research. The Laboratory of Genital Tract Biology is accredited by the College of American Pathologists for immunoassay analysis and operates under strict quality control procedures. SLPI concentrations were measured by the Quantikine enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN), using a Victor2 reader (Perkin Elmer Life Sciences, Boston, MA), as previously described [30, 31]. For initial screening, all samples were diluted 40-fold in the sample diluent provided by R&D Systems, and
all samples showing levels below or above the assay detection range were repeatedly tested at dilutions of 4-fold or 400-fold, respectively, to obtain accurate measurements. All measurements were performed in duplicate. A split quality control pool prepared from cervicovaginal samples was tested on each plate showing an interplate variation of <25%. The total protein concentration in each eluted sample was determined by the BCA assay (Thermo Scientific, Rockford, IL), using the Victor2 counter, and the SLPI concentrations were normalized to milligrams of total protein. For the BCA assay, all samples were screened at a 2-fold dilution in phosphate-buffered saline, and testing was repeated without dilution or at a 5-fold dilution if values were below or above the standard curve, respectively. The coefficient of variation between duplicate values for each sample was <10%.

As the closest surrogate measure of T. vaginalis load available, we used a 4-level grading system based on previously established sensitivities of each T. vaginalis diagnostic test, with the highest load assumed to be corresponding to a positive result wet mount testing (least sensitive test), a moderate load corresponding to a negative result of wet mount testing but positive results of culture and/or antigen testing, and the lowest load corresponding to a positive result of transcription-mediated amplification (TMA) analysis but negative results of all other tests [29, 32, 33]. Theoretically, each of the diagnostic tests can give false-negative results because of testing methods, strain differences, or sample handling. In the literature, the reported false-negative rates are as follows: wet mount testing, 31%–58% [34–36]; culture, 7%–28% [32, 36]; rapid antigen testing, 2%–18% [29, 38]; and TMA analysis, 1%–6% [29, 39, 40]. In addition, the organism load required for a positive test result is reported as 10,000 organism/mL for wet mount testing, 100–500 organisms/mL for culture, 800–2500 organism/mL for rapid antigen testing, and 1–40 organisms/mL for NAAT [41, 42].

Statistical Analyses

Unadjusted Associations

We evaluated the distribution of important covariates by T. vaginalis infection status, using χ² tests for dichotomous variables and analysis of variance (ANOVA) for categorical variables. SLPI results were not normally distributed, so they were log-transformed (natural log) before comparison by means of the Student t test. Differences in mean log SLPI values are presented as the risk ratio (covariate present vs covariate absent), where a value of >1 represents the degree to which the SLPI level is increased, and a value of <1 indicates the degree of decrease when the covariate is present.

Modeling to Adjust for Confounding

In this observational study, there are many potential confounders, such as age, race, sexual behavior, and types of contraception. Failure to adequately consider these confounding effects may bias the results. For example, African American race is overrepresented in the T. vaginalis–infected group and thus may bias the comparison of SLPI levels between the infected and noninfected females. Traditionally, confounding effects are adjusted for by including confounders in the regression model. However, this approach is limited by modeling assumptions, such as distribution and linear additive assumptions, and often requires increases in sample size as the number of confounders increase. The propensity score method is a relatively new technique that does not require the imposition of any distribution or modeling assumptions and helps boost study power. It was proposed to statistically evaluate causal effects free from confounding effects, by mathematically refashioning an observational study into a randomized study; therefore, studies using the propensity score approach are sometimes considered as pseudo-randomized studies [43]. The most commonly used propensity score techniques are matching and inverse weighting, which is considered more appropriate for small sample sizes. Confounders were predetermined on the basis of the current literature and clinical knowledge. We created propensity scores by using both the matching and the inverse weighting technique. Since they gave the same results, we chose to show the weighted results. The distributions of the confounders were compared before and after application of the propensity score methods. For each hypothesis, propensity scores are first created to balance the distribution of confounders between the exposure of interest (present vs absent groups). Then the groups are compared on the basis of their log SLPI values, adjusted by the inverse weight of propensity scores, using a 2-group t test. To adjust for multiple comparisons, we adopted the false-discovery rate (FDR) procedure [44].

For ease of interpretation and to establish normative ranges in adolescent females, we also present the summary statistics in picograms of SLPI per milligram of total protein, derived by calculating the antilog (exponential) of the log-transformed SLPI levels.

RESULTS

Of the 249 females recruited for the original trichomoniasis study, 215 had samples available for SLPI testing and composed the current study sample. The subjects’ characteristics are summarized in Table 1. The mean age was 18.3 years (range, 14–22 years), and the majority self-identified as African American. T. vaginalis was detected (by any T. vaginalis test) in 53 females (24%), C. trachomatis in 49 (22.6%), N. gonorrhoea in 21 (9.7%), and M. genitalium in 30 (14%). Many females had sexual risk factors such as multiple sex partners, a history of prior STI, and a current laboratory-confirmed STI. Females with a positive result of any T. vaginalis test were more likely to have a positive result of an amine test, an abnormal pH, or gonorrhea, compared with those
who were negative for *T. vaginalis*. Of the 53 *T. vaginalis* infections, 20 (38%) had a high load, 27 (50%) had a moderate load, and 6 (11%) had low load. Additionally, 99 (46%) had no past history of *T. vaginalis* infection and no current infection and were considered to be *T. vaginalis* naive; 62 (28.8%) reported prior *T. vaginalis* infection and were currently uninfected, 21 (9.8%) had current infection only, and 32 (14.9%) had both a past history of and current *T. vaginalis* infection (these females were considered to have recurrent infection).

Figure 1 shows the direction and magnitude of each covariate effect on SLPI concentration, unadjusted by propensity score. The bars represent the ratios of mean SLPI concentrations in females with the factor present versus those with the factor absent, illustrating the risk magnitude and direction of change. A risk ratio of less than 1.0 represents a decrease in SLPI level with the factor present, and a risk ratio of greater than 1.0 represents an increase in SLPI level with the factor present. To interpret the risk ratio, a risk ratio of 0.47 means that SLPI levels are decreased by 53%. This value can also be derived by calculating the antilog (exponential) of the difference between the log-transformed levels. SLPI levels were significantly (P < .05, by *t*-tests) lower for those with any hormonal contraception use, a positive result of a *T. vaginalis* test (TMA, culture, antigen, or wet mount), an elevated white blood cell (WBC) count on wet mount testing, and an abnormal vaginal pH of >4.5. There was also a trend for SLPI levels to be higher in females >18 years of age (ratio 1.36) and lower in females with *C. trachomatis* infection (ratio 0.64), but the differences did not reach statistical significance (P > .05 and P < .1, respectively).

We compared mean log SLPI concentration across categorical variables (hormonal contraception use, *T. vaginalis* test results, and *T. vaginalis* infection history), using ANOVA (Table 2). When hormonal contraception was modeled as a dichotomous variable, any hormonal contraception use was associated with a reduced SLPI concentration; however, as a categorical variable, progestin-only contraception appeared to depress the SLPI level more than a combined estrogen-progestin method (ratio 0.61). The SLPI level was significantly lower for females with a high *T. vaginalis* load (positive result of wet mount testing, ratio 0.46) as compared to females with negative results of all *T. vaginalis* tests. Also, recurrent *T. vaginalis* infection appeared to

Table 1. Subject Characteristics, by *Trichomonas vaginalis* (TV) Infection Status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subjects, Proportion (%), by TV Test Result(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥1 Positive</td>
</tr>
<tr>
<td>African American</td>
<td>49/53 (92.5)</td>
</tr>
<tr>
<td>Age ≥18 y</td>
<td>26/53 (49.1)</td>
</tr>
<tr>
<td>History of prior STI</td>
<td>44/53 (83.0)</td>
</tr>
<tr>
<td>Recent sexa</td>
<td>13/53 (24.5)</td>
</tr>
<tr>
<td>Vaginal symptoms present</td>
<td>40/53 (75.5)</td>
</tr>
<tr>
<td>TMA test result</td>
<td></td>
</tr>
<tr>
<td><em>C. trachomatis</em> positive</td>
<td>15/53 (28.3)</td>
</tr>
<tr>
<td><em>N. gonorrhoea</em> positive</td>
<td>10/53 (18.9)</td>
</tr>
<tr>
<td><em>M. genitalium</em> positive</td>
<td>8/53 (15.1)</td>
</tr>
<tr>
<td>High clue cell %</td>
<td>15/53 (28.3)</td>
</tr>
<tr>
<td>Any history of douching</td>
<td>19/53 (35.9)</td>
</tr>
<tr>
<td>Abnormal vaginal pH (&gt;4.5)</td>
<td>35/52 (67.3)</td>
</tr>
<tr>
<td>Hormonal contraception use</td>
<td>17/52 (32.7)</td>
</tr>
<tr>
<td>Clinical BV presenta</td>
<td>7/51 (13.7)</td>
</tr>
<tr>
<td>Multiple sex partners (≥2)</td>
<td>49/51 (96.1)</td>
</tr>
<tr>
<td>Positive result of amine test</td>
<td>34/50 (68.0)</td>
</tr>
<tr>
<td>High WBC countd</td>
<td>12/35 (34.3)</td>
</tr>
<tr>
<td>Sialidase positive (n = 45)</td>
<td>0/5 (0.0)</td>
</tr>
</tbody>
</table>

Column percentages are calculated on the basis of the total number of subjects with a defined characteristic. Denominators differ because of missing data.

Differences were assessed with a *χ*² test.

Abbreviations: BV, bacterial vaginosis; *C. trachomatis*, Chlamydia trachomatis; *M. genitalium*, Mycoplasma genitalium; *N. gonorrhoea*, Neisseria gonorrhoea; STI, sexually transmitted infection; TMA, transcription-mediated amplification; WBC, white blood cell.

a Within the past 2 days.

b Defined as >20% of epithelial cells per field.

c Defined as an abnormal vaginal pH (>4.5), a high clue cell percentage (>20% of epithelial cells per field), and a positive result of an amine test.

d Defined as ≥6 cells/high-power field on wet mount.
depress SLPI levels (ratio 0.49) to a greater degree than first-time (current only) *T. vaginalis* infection.

Figure 2 shows the effect of propensity weighting on the distribution of several important covariates, compared with *T. vaginalis* infection status. Variables displayed are those known to affect either *T. vaginalis* infection status or SLPI level. The y-axis shows the difference in the percentage of females who were *T. vaginalis* positive, compared with those who were *T. vaginalis* negative, before (light bar) and after (dark bar) propensity weighting. For example, before weighting, the proportion of females who self-reported African American race was higher (by about 10%) among those who were *T. vaginalis* positive as compared to those who were *T. vaginalis* negative, before (light bar) and after (dark bar) propensity weighting. For example, before weighting, the proportion of females who self-reported African American race was higher (by about 10%) among those who were *T. vaginalis* positive as compared to those who were *T. vaginalis* negative; after weighting, the difference was only about 4%. Although the differences prior to weighting were significant only for *N. gonorrhoea* infection and a positive result of amine testing, all covariates became more evenly distributed after weighting, with no significant differences. In addition to analyzing differences on the basis of *T. vaginalis* infection status (Figure 2), we also examined the effect of weighting on the distribution of covariates with respect to factors found to be associated with the SLPI level in our data set (ie, abnormal vaginal pH, high WBC count on wet mount testing, and history of *T. vaginalis* infection; data not shown). In each case, propensity weighting removed the imbalance of variables and resulted in a more even distribution of covariates.

Table 3 shows the results obtained after propensity weighting. After weighting, the SLPI concentration was not associated with the combined variable “positive result of any *T. vaginalis* test.” The SLPI level was decreased in females with a positive result of the *T. vaginalis* antigen test (ratio 0.64), an elevated vaginal pH (ratio 0.6), and vaginal leukocytosis (defined as >6 WBCs/HPF on wet mount testing; ratio 0.44). Compared with females with an initial/current infection, females with recurrent *T. vaginalis* infection had a significantly depressed SLPI level (ratio 0.49). Among females with a positive result of any *T. vaginalis* test, those with a positive result of wet mount testing had the lowest SLPI level (ratio 0.27), compared with those with a negative result of wet mount testing. After control by use of the FDR procedure, only the *T. vaginalis* antigen test became marginally nonsignificant (adjusted *P* = .058 vs unadjusted *P* = .048); the rest of the results remained the same.

---

**Figure 1.** Direction and magnitude of covariate effect on the secretory leukocyte protease inhibitor (SLPI) level, unadjusted by propensity score. The bars represent the risk ratios of SLPI concentrations (log10 of pg of SLPI per mg of total protein) in women with the specified factor present vs those with that factor absent. A ratio of <1.0 represents a decrease in SLPI level with the factor present, and a ratio of >1.0 represents an increase in SLPI level with the factor present. Solid dark bars are significant at *P* < .05, striped bars have borderline significance (*P* > .05 and ≤ .1), and light bars are not significant (*P* > .1, by the *t* test). aDefined as >20% of epithelial cells per field. bDefined as an abnormal vaginal pH (>4.5), a high clue cell percentage (>20% of epithelial cells per field), and a positive result of an amine test. cDefined as >6 cells/high-power field on wet mount. Abbreviations: Ag, antigen; BV, bacterial vaginosis; *C. trachomatis*, Chlamydia trachomatis; STD, sexually transmitted disease; TMA, transcription-mediated amplification; *T. vaginalis*, Trichomonas vaginalis; WBC, white blood cell.

---

Table 3

<table>
<thead>
<tr>
<th>Covariate</th>
<th>SLPI Level (Ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive result of any <em>T. vaginalis</em> test</td>
<td>0.64</td>
</tr>
<tr>
<td>Elevated vaginal pH</td>
<td>0.6</td>
</tr>
<tr>
<td>Vaginal leukocytosis (defined as &gt;6 WBCs/HPF on wet mount testing)</td>
<td>0.44</td>
</tr>
<tr>
<td>Recurrent <em>T. vaginalis</em> infection</td>
<td>0.49</td>
</tr>
</tbody>
</table>

---

*Abbreviations: Ag, antigen; BV, bacterial vaginosis; *C. trachomatis*, Chlamydia trachomatis; STD, sexually transmitted disease; TMA, transcription-mediated amplification; *T. vaginalis*, Trichomonas vaginalis; WBC, white blood cell.*
We have generated solid evidence from these cross-sectional data that a depressed SLPI concentration is highly associated with T. vaginalis infection, as determined by a positive result of rapid antigen testing and wet mount testing. We have previously shown that the rapid antigen test is comparable to culture and more sensitive than wet mount testing [29]. In addition, the antigen test is clinically available, but it has not yet been widely used to assess the risk of outcomes or response to treatment for T. vaginalis.

Our data suggest that the mucosal immune defense is impaired by T. vaginalis in a T. vaginalis load–dependent manner. Among adolescent and young adult females with any evidence of T. vaginalis infection, the SLPI concentration was 73% lower (risk ratio 0.27) in those with positive results of wet mount testing, compared with those with negative results of wet mount testing but positive results of antigen testing, culture, or TMA analysis. However, it is unknown whether identifying and treating women with positive results of NAAT but negative results of wet mount tests or culture (it is presumed that such individuals have low levels of infection) will prevent the decrease in SLPI concentration. This knowledge should be gained through a clinical trial and would be highly useful in determining the appropriateness of asymptomatic T. vaginalis screening, as well as for preventing SLPI-mediated HIV transmission.

It is well known that women with 1 episode of T. vaginalis infection are at high risk for recurrent and persistent infections [45, 46]. A recurrent infection may be facilitated by lower SLPI levels. This hypothesis is in agreement with our finding that the SLPI level was approximately 50% lower among females with recurrent T. vaginalis infection than in those with an initial infection. This is similar to our understanding of the immune response to C. trachomatis infection, in which subsequent infections induce a brisker inflammatory response that damages the host, compared with an initial infection. If these cross-sectional data are confirmed in longitudinal studies, it might lead us to develop a “prevention for positives” approach to the control of trichomoniasis. For example, it may be more cost-effective to develop strategies to prevent future complications among women who test positive for a first episode of trichomoniasis than to prevent the first infection.

Another possible explanation for lower SLPI levels in women with recurrent infection is that some of those women could have a persistent, rather than recurrent, T. vaginalis infection that may not be responding to treatment. A number of recent studies suggest that failed treatment may be more common than was previously reported [45, 47]. We have
recently shown that *Trichomonas vaginalis* can directly suppress SLPI expression via its surface lipophosphoglycan [31].

Finally, we confirmed that traditional markers of vaginal barrier disruption, such as elevated vaginal pH and leukocytosis, are strongly associated with decreases in SLPI concentration and, thus, are markers of impaired vaginal immune defense. This knowledge could have 2 uses. With others, we have shown that women can accurately assess their vaginal pH by using an over-the-counter device and then seek medical care to determine the presence of bacterial vaginosis or *T. vaginalis* infection, especially if they have engaged in risky behavior [21, 48]. Second, our findings support prior studies suggesting that the SLPI level should be used to evaluate the safety of vaginal products, ranging from tampons to microbicides [49, 50].

**Table 3. Effect of Variables on Secretory Leukocyte Protease Inhibitor (SLPI) Levels After Propensity Score Weighting**

<table>
<thead>
<tr>
<th>Variable, Response</th>
<th>Subjects, No.</th>
<th>SLPI Level, Mean&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Log</th>
<th>Antilog</th>
<th>RR(95% CI)</th>
<th>P&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any TV test result</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>53</td>
<td>10.92</td>
<td>55271</td>
<td>.79 (.51–1.21)</td>
<td>.27</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>162</td>
<td>11.16</td>
<td>70263</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TV antigen test result</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>47</td>
<td>10.74</td>
<td>46166</td>
<td>.64 (.41–.95)</td>
<td>.048</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>168</td>
<td>11.2</td>
<td>73130</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal (&gt;4.5)</td>
<td>93</td>
<td>10.85</td>
<td>51534</td>
<td>.60 (.40–.89)</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Normal (≤4.5)</td>
<td>116</td>
<td>11.36</td>
<td>85819</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count on wet mount</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (&gt;6 cells/HPF)</td>
<td>42</td>
<td>10.44</td>
<td>34201</td>
<td>.44 (.28–.70)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Normal (≤6 cells/HPF)</td>
<td>123</td>
<td>11.26</td>
<td>77653</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TV history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naive</td>
<td>99</td>
<td>11.1</td>
<td>66171</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past only</td>
<td>62</td>
<td>11.23</td>
<td>75358</td>
<td>.83 (.56–1.37)</td>
<td>.56&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Current only</td>
<td>21</td>
<td>11.29</td>
<td>80017</td>
<td>.94 (.51–1.73)</td>
<td>.84&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Both past and current</td>
<td>32</td>
<td>10.58</td>
<td>39340</td>
<td>.49 (.30–.91)</td>
<td>.03&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Wet mount result&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>20</td>
<td>9.8</td>
<td>18034</td>
<td>.27 (.11–.68)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>33</td>
<td>11.1</td>
<td>66171</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis is limited to significant variables. Each row represents a separate propensity analysis. Abbreviations: CI, confidence interval; HPF, high-power field; RR, risk ratio; TV, *Trichomonas vaginalis*; WBC, white blood cell.

<sup>a</sup> Log values are propensity score weighted. Antilog values are pg/mg protein and are provided to facilitate interpretation.
<sup>b</sup> Calculated as [log<sub>10</sub> of SLPI level in pg/mg total protein] in women with the factor present vs those with the factor absent.
<sup>c</sup> Two-group *t* test adjusted by inverse weighting of propensity scores.
<sup>d</sup> Compared with naive.
<sup>e</sup> Compared with past only.
<sup>f</sup> Compared with current only.
<sup>g</sup> Data are for women with any positive TV test result (n = 53).
concentrations may change over time with the duration of infection. With this data set, we could not determine the duration of any *T. vaginalis* infections. In addition, we could only estimate organism load on the basis of the type of *T. vaginalis* test yielded positive results. However, our approach is reasonable, given that others have shown by real-time, semiquantitative PCR that culture-positive specimens have a >2 log10 higher concentration of DNA than specimens that are PCR positive but culture negative [42]. Some infections that we labeled as “probable recurrent infections” might have actually represented persistent infections due to failure of prior treatments [45].

In conclusion, in adolescent and young adult females, a depressed SLPI level is strongly associated with *T. vaginalis* infection, bacterial vaginosis, hormonal contraceptive use, and younger age. Further studies should evaluate the SLPI level as a promising marker of the vaginal immune response to *T. vaginalis*, bacterial vaginosis, and other STIs in nonpregnant and pregnant females of all ages.

**Notes**

**Financial support.** This work was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH; grant 5K23AI063182-04 to J. S. H. and grant 5R01AI079853-04 to R. N. F.), and the National Institute of Biomedical Imaging and Bioengineering, NIH (grant 1U54EB007958-01 to J. S. H.).

**Potential conflicts of interest.** J. S. H. has received honorarium from Genzyme/Sekisui as a speaker. All other authors report no 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**