Vaginal leucocyte counts in women with bacterial vaginosis: relation to vaginal and cervical infections

W M Geisler, S Yu, M Venglarik, J R Schwebke

Objective: To evaluate whether an elevated vaginal leucocyte count in women with bacterial vaginosis (BV) predicts the presence of vaginal or cervical infections, and to assess the relation of vaginal WBC counts to clinical manifestations.

Methods: We retrospectively analysed the relation of vaginal leucocyte counts to vaginal and cervical infections and to clinical manifestations in non-pregnant women diagnosed with BV at an STD clinic visit. Results: Of 296 women with BV studied, the median age was 24 years and 81% were African-American. Elevated vaginal leucocyte counts were associated with objective signs of vaginitis and cervicitis and also predicted candidiasis (OR 7.9, 95% CI 2.2 to 28.9), chlamydia (OR 3.1, 95% CI 1.4 to 6.7), gonorrhoea (OR 2.7, 95% CI 1.3 to 5.4), or trichomoniasis (OR 3.4, 95% CI 1.6 to 7.3). In general, as a screening test for vaginal or cervical infections, vaginal leucocyte count had moderate sensitivities and specificities, low positive predictive values, and high negative predictive values.

Conclusions: An elevated vaginal leucocyte count in women with BV was a strong predictor of vaginal or cervical infections. Vaginal leucocyte quantification may provide an alternative approach to assessing need for empirical therapy for chlamydia and gonorrhoea, particularly in resource-limited high STD risk settings that provide syndromic management.

Bacterial vaginosis (BV) is a common disorder of the genital tract in women characterised by an alteration of a normal acidic lactobacilli predominant vaginal ecosystem to a vaginal environment dominated by organisms such as Gardnerella vaginalis, Mycoplasma sp, and anaerobes, with an accompanying increase in pH. BV has been associated with upper genital tract infection, and has been reported to be a strong predictor of chlamydia and gonococcal cervicitis. While diagnosis of BV is based on reliable established criteria, diagnosis of cervical infection is more problematic and the significant medical and economic implications of untreated chlamydia or gonococcal infection influence empirical approaches to these disorders.

Although BV has traditionally been considered a non-inflammatory syndrome without an association with increased vaginal leucorrhoea, several studies have demonstrated the presence of vaginal leucocytes (white blood cells, WBCs) in women with bacterial vaginosis. Recent studies have reported an association of BV with elevated inflammatory cytokines. Some studies have reported an association of vaginal WBCs in women who have BV with concomitant chlamydial or gonococcal cervicitis or histological endometritis, though possible limitations in some of these studies may have influenced the validity of the findings. Such limitations include small sample size, provider inexperience, having multiple providers, use of a vaginal WBC count definition with lower sensitivity, and not addressing concomitant vaginal infections. There is also a paucity of studies characterising the relation of vaginal WBC counts to clinical manifestations of vaginal and cervical infections.

An improved understanding of the relation of vaginal WBC counts to vaginal and cervical infections, along with associated clinical manifestations of these diseases, would be important in the assessment of risk for sexually transmitted diseases (STDs) and need for empirical therapy, particularly for chlamydia and gonorrhoea, in a resource limited setting with a high prevalence of STDs that may practise syndromic management because of insufficient means to further test for these pathogens. Addressing limitations of previous related studies, we evaluated whether an elevated vaginal WBC count in women with BV diagnosed at a routine first time STD clinic visit predicted the presence of vulvovaginal candidiasis (VVC) or STDs, and we explore the relation of vaginal WBC counts to clinical manifestations of such diseases. Further, we assess how the finding of mucopurulent cervical discharge compares to vaginal WBC count in predicting chlamydia and gonococcal infections.

METHODS

Study population and data collection

Through a computerised databank, we identified all women who were seen by a single, experienced provider for a routine first time visit from 1998 through 2002 at the Jefferson County Department of Public Health (JCDH)-STD Clinic and who were diagnosed with BV at that visit. BV affects a significant proportion of women presenting to the JCDH-STD Clinic. A routine visit consisted of data collection and laboratory testing for either screening for STDs or evaluation of a specific complaint. Individuals presenting for treatment of a laboratory confirmed STD, those pregnant, women with a previous hysterectomy, and those with missing data relative to vaginal WBC counts or the diagnoses of BV, VVC, or concomitant STDs were excluded.

Demographic, clinical, and laboratory data (relevant to vaginal WBC counts and clinical disease) on study subjects were retrospectively collected from the JCDH-STD Clinic computerised database. Laboratory data routinely collected included vaginal WBC counts and results of evaluation for BV, chlamydia, gonorrhoea, trichomoniasis, and VVC. Vaginal WBCs were previously quantified after visualisation of a minimum of five fields (range 5–15 based on whether there was a paucity of WBCs present) under light microscopy at ×400. Vaginal WBC counts were routinely categorised into

Abbreviations: BV, bacterial vaginosis; OCP, oral contraceptive pill; VVC, vulvovaginal candidiasis; WBCs, white blood cells

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either ≤5 WBCs per ×400 in all visualised fields (representing minimal or no inflammation) or >5 WBCs per ×400 in at least one field visualised (considered elevated and more suggestive of significant inflammation). During the study period, the diagnosis of BV was routinely made based on the criteria of Amsel et al10 and required the presence of three of the following findings: (1) vaginal pH >4.5; (2) visualisation of clue cells by light microscopy at ×400; (3) a positive whiff test on application of 10% potassium hydroxide to vaginal fluid; or (4) homogeneous vaginal discharge. Chlamydia trachomatis was detected by culture of an endocervical swab specimen using DEAE-pretreated McCoy cells on 96-well microtitre plates and identification of chlamydial inclusions as previously described.11 Neisseria gonorrhoeae was detected by culture of an endocervical swab specimen on modified Thayer-Martin medium using a standard method12 or by Gonostat (Sierra Diagnostics, Inc, Sonora, CA, USA). Trichomoniasis was detected in most women by visualisation of trichomonads on a vaginal wet preparation by light microscopy at ×400. Some women, who were being screened for future enrolment in other research studies, also had additional testing by culture for Trichomonas vaginalis in modified Diamond’s media as previously described13; proportion of individuals receiving culture did not differ by vaginal WBC count category. VVC was diagnosed based on the presence of vaginal discharge on examination and visualisation of budding yeast or pseudohyphae on a vaginal wet preparation by light microscopy at ×400. The diagnosis of genital herpes was based on characteristic clinical findings. The study was approved by the institutional review boards of the University of Alabama at Birmingham and JCDH.

Statistical analyses were conducted on Stata (Stata Corp, Release 6.0, College Station, TX, USA). Significance of differences in demographic and clinical characteristics by leucocyte count category was determined by univariate logistic regression or Fisher’s exact test, with the exception of the co-variates age, which was non-normally distributed and therefore its association was analysed by the Wilcoxon rank-sum (Mann-Whitney) test. The relation of vaginal leucocyte count in women with BV to VVC and STDs was assessed through univariate regression and then through a multivariate regression controlling for age, oral contraceptive pill (OCP) use, VVC, gonorrhoea, chlamydia, trichomoniasis, and genital herpes. Age and OCP use were included in the multivariate model a priori as they were considered to be potential confounders; age data were transformed into the natural log. The sensitivity, specificity, and predictive values of vaginal WBC count for the diagnosis of VVC or an STD were calculated by using 2 × 2 tables. To assess correlation between vaginal WBC count and the finding of mucopurulent cervical discharge, a correlation coefficient was determined. The relation of either mucopurulent cervical discharge alone or combined with vaginal WBC count to cervical chlamydia and gonorrhoea was assessed through multivariate analysis controlled for co-variates as described above.

RESULTS

Characteristics of subjects and relation to vaginal leucocyte count

From 1998 through 2002, 296 women seen for a routine first time visit at the JCDH-STD Clinic were diagnosed with BV and were eligible for study. The median age was 24 years (range 14–61). Eighty one per cent of women were African-American and 19% were of other racial/ethnic backgrounds (predominately white). Sixteen per cent of women used OCPs for contraception. The most frequent symptom reported was vaginal discharge (46%), with vaginal odour (28%), genital itching (15%), lower abdominal pain (13%), and dysuria (7%) being reported less frequently. The most common examination finding was abnormal vaginal discharge (87%), with vaginal erythema (20%), mucopurulent cervical discharge (16%), cervical friability (10%), adnexal tenderness (6%), cervical motion tenderness (4%), fundal tenderness (4%), and abdominal tenderness (3%) being found less commonly.

Demographic and clinical characteristics of the study population stratified by vaginal WBC count category are presented in table 1. Vaginal WBC count was elevated in 125 (42%) women. Age, race, OCP use, and symptoms reported did not significantly differ by vaginal WBC count category. Women with an elevated vaginal WBC count were significantly more likely to present with objective evidence of vaginitis (abnormal vaginal discharge, p<0.0001; vaginal erythema, p = 0.008) and cervicitis (mucopurulent cervical discharge, p<0.0001; cervical friability, p = 0.10).

Table 1 Demographic and clinical characteristics of women with bacterial vaginosis, stratified by vaginal leucocyte count (WBC per ×400)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WBC &lt;5 (n=171)</th>
<th>WBC ≥5 (n=125)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (years) (range)</td>
<td>25 (14–61)</td>
<td>23 (14–48)</td>
<td>0.39</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>139 (81)</td>
<td>100 (80)</td>
<td>0.78</td>
</tr>
<tr>
<td>Other</td>
<td>32 (19)</td>
<td>25 (20)</td>
<td>–</td>
</tr>
<tr>
<td>OCP use</td>
<td>23 (14)</td>
<td>24 (19)</td>
<td>0.18</td>
</tr>
<tr>
<td>Reported abdominal pain</td>
<td>19 (11)</td>
<td>18 (14)</td>
<td>0.40</td>
</tr>
<tr>
<td>Reported dysuria</td>
<td>13 (8)</td>
<td>8 (6)</td>
<td>0.69</td>
</tr>
<tr>
<td>Reported vaginal odour</td>
<td>48 (28)</td>
<td>34 (27)</td>
<td>0.87</td>
</tr>
<tr>
<td>Reported genital itching</td>
<td>28 (16)</td>
<td>16 (13)</td>
<td>0.39</td>
</tr>
<tr>
<td>Reported vaginal discharge</td>
<td>73 (43)</td>
<td>63 (50)</td>
<td>0.19</td>
</tr>
<tr>
<td>Vaginal discharge finding</td>
<td>127 (80)</td>
<td>120 (96)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vaginal erythema</td>
<td>22 (15)</td>
<td>24 (27)</td>
<td>0.008</td>
</tr>
<tr>
<td>Cervical friability</td>
<td>12 (7)</td>
<td>16 (13)</td>
<td>0.10</td>
</tr>
<tr>
<td>Mucopurulent cervical discharge</td>
<td>10 (6)</td>
<td>36 (29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cervical motion tenderness</td>
<td>2 (1)</td>
<td>10 (8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Fundal tenderness</td>
<td>4 (2)</td>
<td>7 (6)</td>
<td>0.16</td>
</tr>
<tr>
<td>Adnexal tenderness</td>
<td>8 (5)</td>
<td>11 (9)</td>
<td>0.16</td>
</tr>
<tr>
<td>Abdominal tenderness</td>
<td>2 (1)</td>
<td>8 (6)</td>
<td>0.03</td>
</tr>
</tbody>
</table>
with an elevated vaginal WBC count were also significantly more likely to present with some objective evidence of upper genital tract disease (cervical motion tenderness, p = 0.01; abdominal tenderness, p = 0.03), though some objective findings did not significantly differ by vaginal WBC count category (fundal tenderness, p = 0.16; adnexal tenderness; p = 0.16).

**Frequency of vaginal and cervical infections and relation to vaginal leucocyte count**

VVC and genital herpes were diagnosed in 6% and 3% of women, respectively. *C trachomatis* and *N gonorrhoeae* cervical infection was detected in 15% and 16% of women, respectively. Trichomoniasis was identified in 14% of women. Stratified by vaginal WBC count, women with an elevated vaginal WBC count were significantly more likely on univariate analysis to have VVC (OR 7.6, 95% CI 2.2 to 27.0, p = 0.002), chlamydia (OR 3.4, 95% CI 1.7 to 6.7, p<0.0001), gonorrhoea (OR 2.9, 95% CI 1.5 to 5.5, p = 0.001), or trichomoniasis (OR 3.1, 95% CI 1.5 to 6.2, p = 0.001). The proportion of women with VVC or STDs stratified by vaginal WBC count category and significance of these relations by multivariate analysis is presented in table 2. Controlling for age, OCP use, and concomitant VVC or STDs, the statistical association of an elevated vaginal WBC count with vaginal and cervical infections remained just as highly significant. Genital herpes was not associated with vaginal WBC count on univariate or multivariate analysis, but was highly significant. Genital herpes was not associated with chlamydia (17% v 14%, p = 0.72).

Elevated vaginal WBC counts significantly correlated with the finding of mucopurulent cervical discharge (R² = 0.31; p<0.001). On multivariate analysis, the combined finding of elevated vaginal WBC count and mucopurulent cervical discharge still significantly predicted cervical gonococcal infection (36% v 13%, OR 3.7, 95% CI 1.6 to 8.2; p = 0.002), but in contrast with elevated vaginal WBC count alone, did not significantly predict cervical chlamydial infection (22% v 14%, p = 0.36).

**Comparison of mucopurulent cervical discharge finding to vaginal leucocyte count in predicting vaginal and cervical infections**

For comparison with vaginal WBC counts, we assessed whether the presence of mucopurulent cervical discharge also independently predicted the presence of cervicitis due to chlamydia or gonorrhoea. As found with elevated vaginal WBC counts on multivariate analysis, the presence of mucopurulent cervical discharge was significantly associated with gonorrhoea (33% v 13%, OR 3.5, 95% CI 1.7 to 7.4, p = 0.001). In contrast with vaginal WBC counts, the presence of mucopurulent cervical discharge was not significantly associated with chlamydia (17% v 14%, p = 0.72).

**DISCUSSION**

Detection of vaginal WBCs is a simple, inexpensive means to assess for inflammation in the vaginal or cervical tissue and may serve as an important marker for underlying infection. The presence of vaginal WBCs has previously been shown to be a predictor of infectious cervicitis and upper genital tract infection. Although BV has traditionally been considered a non-inflammatory syndrome without an association with increased vaginal leucorrhoea, several studies have demonstrated the presence of vaginal WBCs in women with BV and recent studies have reported an association of BV with elevated inflammatory cytokines. It is possible that the vaginal microbial flora present in BV, independent of concomitant vaginal or cervical infections, may induce an inflammatory response.

**Performance characteristics of vaginal leucocyte count as a screening test**

The diagnostic test characteristics of vaginal WBC count for predicting VVC or STDs are presented in table 3. In general, the sensitivities and specificities of vaginal WBC count for diagnosing VVC or STDs were moderate; the positive predictive values of this screening test were low, but the negative predictive values were high (especially for VVC).

**Table 2** Multivariate model assessing the relation of an elevated vaginal leucocyte count (WBC per x400) in women with bacterial vaginosis to vaginal or cervical infections

<table>
<thead>
<tr>
<th>Predictor</th>
<th>WBC ≤ 5 (n = 171)</th>
<th>WBC &gt; 5 (n = 125)</th>
<th>OR (95% CI)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (years) (range)</td>
<td>25 (14–61)</td>
<td>23 (14–48)</td>
<td>0.9 (0.4 to 2.3)</td>
<td>0.85</td>
</tr>
<tr>
<td>OCP use</td>
<td>23 (14)</td>
<td>24 (19)</td>
<td>1.4 (0.7 to 2.8)</td>
<td>0.32</td>
</tr>
<tr>
<td>Vulvovaginal candidiasis</td>
<td>3 (2)</td>
<td>15 (12)</td>
<td>7.9 (22.2 to 28.9)</td>
<td>0.002</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>14 (8)</td>
<td>27 (22)</td>
<td>3.4 (1.6 to 7.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cervical chlamydia</td>
<td>14 (8)</td>
<td>29 (23)</td>
<td>3.1 (1.4 to 6.7)</td>
<td>0.004</td>
</tr>
<tr>
<td>Cervical gonorrhoea</td>
<td>17 (10)</td>
<td>30 (24)</td>
<td>2.7 (1.3 to 5.4)</td>
<td>0.005</td>
</tr>
<tr>
<td>Genital herpes</td>
<td>6 (4)</td>
<td>2 (2)</td>
<td>0.7 (0.1 to 3.7)</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*Odds ratios, 95% confidence intervals, and p values were determined by multivariate regression controlling for age (log transformed), OCP use, vulvovaginal candidiasis, and genital chlamydia, gonorrhoea, and herpes.

**Table 3** Sensitivity, specificity, and predictive values of vaginal leucocyte count for predicting vaginal or cervical infections in women with bacterial vaginosis

<table>
<thead>
<tr>
<th></th>
<th>VVC*</th>
<th>Chlamydia</th>
<th>Gonorrhoea</th>
<th>Trichomoniasis</th>
<th>Any infection**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>83</td>
<td>67</td>
<td>64</td>
<td>66</td>
<td>65</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>60</td>
<td>62</td>
<td>62</td>
<td>62</td>
<td>72</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>12</td>
<td>23</td>
<td>24</td>
<td>22</td>
<td>59</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>98</td>
<td>92</td>
<td>90</td>
<td>92</td>
<td>77</td>
</tr>
</tbody>
</table>

*Vulvovaginal candidiasis.

**Positive for VVC, chlamydia, gonorrhoea, or trichomoniasis.
use of vaginal WBC counts as a screening test in settings with readily available access to more sensitive and specific chlamydial and gonococcal assays, there is a potential role for this screening test in assessing STD risk and need for empiric therapy in more resource limited high STD risk settings which rely predominately on syndromic management for cervical infections. In our study, we found that reported symptoms were not associated with vaginal WBC count. Ryan et al found a chief complaint of vaginal discharge actually predicted a lower rate of gonorrhea or chlamydial infection compared with rates observed in patients with no such complaint. They also found that the addition of specimen and bimanual examinations as well as microscopy to an algorithm of risk assessment and symptom review increased the specificity and positive predictive value for chlamydial and gonococcal infection. Given the limited ability of syndromic management algorithms to predict those with chlamydial or gonococcal infections and the potential for such algorithms to lead to overtreatment of a significant portion of uninfected individuals, vaginal WBC measurements, with high negative predictive value for chlamydia and gonorrhea, could provide an alternative non-invasive method (compared with addition of speculum examination) for screening for chlamydia and gonorrhea in such settings.

The finding of mucopurulent discharge correlated significantly with an elevated vaginal WBC count in our study and was a predictor of cervical gonococcal infection. In contrast with vaginal WBC count, mucopurulent discharge did not predict cervical chlamydial infection. Other studies have also reported that neither mucopurulent discharge nor endocervical swab test strongly predicted chlamydial infection. It is plausible that chlamydial infection may induce only a minimal inflammatory response, which has been demonstrated in men, and microscopic measurement of inflammation as was done with vaginal WBC counts in our study may provide a more sensitive measure for predicting chlamydial infection. Use of a more sensitive cut-off value for elevated vaginal WBC count in our study (˃5 WBCs per ×400) may have increased the likelihood that vaginal WBC count would predict chlamydial infection.

The retrospective design of this study is a limitation that may influence clinical and laboratory data. The number of fields counted during vaginal WBC quantitation varied depending on the paucity or abundance of WBCs, though since WBC counts were categorised by a cut-off value rather than total number, this probably had minimal influence on the relations studied. The chlamydial and gonococcal assays that had been utilised in our study population are not the most sensitive assays available, which may confound the association of vaginal WBC counts with cervical infections; use of more sensitive nucleic acid amplification tests may identify a few more cases of cervical chlamydial or gonococcal infections and may aid in more accurately evaluating vaginal WBC counts as a screening tool for cervical infections. It is unclear whether findings from our study could be extrapolated to populations with a lower incidence of vaginal or cervical infections. The higher risk STD clinic population in this study may be more likely to have had previous vaginal or cervical infections, in which partial cell or humoral mediated immune responses could attenuate the inflammatory response; if this were the case, then the association of vaginal WBC counts with vaginal or cervical infections in women with BV may actually be stronger in an STD naive population. Reliable data on STD history were not available in these patients.

It remains unclear whether inflammation is induced by the vaginal microbes present in BV in the absence of concomitant vaginal or cervical infections. Use of more sensitive assays for routinely tested vaginal and cervical pathogens in future
studies may help clarify this issue, as would attempting to identify subpopulations of women with BV, by Nugent score classification or quantitation of vaginal microbes, which may be more likely to have vaginal inflammation. Other directions in improving our understanding of the relation of vaginal WBCs to BV may include measuring for other potential vaginal or cervical pathogens, such as Mycoplasma or Ureaplasma, that may induce inflammation but that are not routinely tested. A better understanding of the relation of vaginal WBCs to VVC and STDs in women with BV has important implications in assessment of STD risk and need for empirical therapy in certain settings.

CONTRIBUTORS
WG and MV conceived and designed the study; WG performed the data analyses; SY extracted data from the databank and assisted in data analyses; WG and JS drafted the manuscript; SY and MV reviewed the manuscript.

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