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PELVIC INFLAMMATORY DISEASE SUPPLEMENT

Bacterial Vaginosis and Behavioral Factors Associated With Incident Pelvic Inflammatory Disease in the Longitudinal Study of Vaginal Flora

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Background. Pelvic inflammatory disease (PID) leads to long-term reproductive consequences for cisgender women. Bacterial vaginosis (BV) and behavioral factors may play a role in PID pathogenesis. We assessed associations between BV, behavioral factors, and incident PID.

Methods. We analyzed participants (N = 2956) enrolled in the National Institutes of Health Longitudinal Study of Vaginal Flora, a cohort of nonpregnant cisgender women followed quarterly for 12 months. PID was defined by at least 1 of the following: cervical motion tenderness, uterine tenderness, or adnexal tenderness (160 cases). We tested associations between BV (measured using Nugent and Amsel criteria) and PID at the subsequent visit. Sociodemographic factors, sexual behaviors, and *Chlamydia trachomatis* (CT), untreated at baseline and concurrent with BV, were covariates in Cox proportional hazards models. Adjusting for the few *Neisseria gonorrhoeae* and *Trichomonas vaginalis* cases did not alter results.

Results. In multivariable modeling, Nugent-BV (adjusted hazard ratio [aHR], 1.53 [95% confidence interval {CI}, 1.05–2.21]), symptomatic Amsel-BV (aHR, 2.15 [95% CI, 1.23–3.75]), and vaginal douching (aHR, 1.47 [95% CI, 1.03–2.09]) were associated with incident PID.

Conclusions. BV was associated with incident PID in a large prospective cohort, controlling for behavioral factors and sexually transmitted infections (STIs). Larger studies on how BV, STIs, behaviors, and host responses interactively affect PID risk are needed.

Keywords. pelvic inflammatory disease; bacterial vaginosis; cohort.

Pelvic inflammatory disease (PID) represents a spectrum of inflammatory disorders of the upper female reproductive tract [1], which can lead to severe long-term reproductive consequences including tubal factor infertility, ectopic pregnancy, and chronic pelvic pain [1–6]. Despite some recent declines in PID rates, it remains common, affecting an estimated 4.4% of reproductive-aged cisgender women in the United States, with an estimated 2.5 million prevalent cases nationwide and direct treatment costs over $2 billion annually [7]. Infertility caused by PID can also affect the emotional and mental wellness of couples seeking children. PID may result from microorganisms that ascend from the vagina or cervix to infect upper genital tract structures. Sexually transmitted infections (STIs) including *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Mycoplasma genitalium* (MG) are implicated in a substantial proportion of cases [3–5, 8–14], while behaviors such as vaginal douching have also been associated with an increased risk of PID [15, 16]. Bacterial vaginosis (BV), a clinical condition characterized by a vaginal microbiota low in *Lactobacillus* species and dominated by facultative and anaerobic bacteria, may play a role in PID pathogenesis as well [17, 18].

Upper tract structures can be directly infected by NG, CT, and MG; these organisms may also facilitate the ascension of BV-associated organisms from the lower genital tract, presumably due to breakdown of barriers and immune defense mechanisms in the setting of inflammation [3–5, 8–14]. BV-associated bacteria are commonly found in the upper genital tract of women with PID including those in which an STI was not identified [19]. Soper et al reported vaginal microorganisms were isolated from the endometrium in 31.4% and from the cul-de-sac in 14.3% of women with acute salpingitis [20]. BV organisms have been shown in model systems to cause oviduct damage [21]. Additionally, BV has been associated with increased risk of STI acquisition [22–25]. BV may contribute to PID by increasing susceptibility to and decreasing clearance of STIs, or through synergistic relationships whereby BV-associated bacteria facilitate the ascension of STIs [21]. As up to 70% of PID cases are not linked to a known STI [14], an

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active area of inquiry is whether BV can independently (in the absence of STIs) induce PID [21, 26].

Currently, data on the role of BV in PID are sparse. Some, but not all, studies show a positive association between BV and PID (see review by Taylor et al) [16, 20, 21, 26–40]. The majority of studies have been cross-sectional (assessing BV at the time of PID diagnosis), and many did not control for STIs such as CT or NG [21]. Few studies have prospectively assessed BV and PID or examined BV status antecedent to incident PID [16, 26, 28, 29].

The Centers for Disease Control and Prevention and other national and professional organizations recommend annual CT and NG screening for all sexually active women in the United States under age 25 [1, 41]. Yet, PID remains common and new methods to prevent PID are needed. Novel therapeutics to treat BV and optimize the vaginal microbiota are currently under development [42]. If antecedent BV can be shown to increase risk of PID (irrespective of whether this relationship is mediated via new STI acquisition), this would support the need for further studies to elucidate mechanisms and, perhaps in the long term, to trial vaginal microbiota modification as a way to prevent PID. To date, we lack prospective, well-controlled data on the relationship between BV and PID. Here, we sought to assess whether antecedent BV is associated with incident PID diagnosis in a large prospective cohort.

METHODS

Parent Study

The National Institutes of Health Longitudinal Study of Vaginal Flora (LSVF) was conducted from August 1999 to February 2002 and recruited 3620 nonpregnant cisgender women (aged 15–44 years) through clinics in Birmingham, Alabama [43]. Exclusion criteria included immunocompromised status, hysterectomy, menopause, pelvic radiotherapy, antibiotic therapy >30 days, nonfluency in English, plans to move within the next 12 months, participation in a clinical trial using antibiotics or genital microbicides, and limitations preventing informed consent. Participants were followed quarterly for approximately 12 months with clinical examinations and surveys. All participants gave written informed consent, and the study was approved by the institutional review boards of the National Institute of Child Health and Human Development, the Jefferson County Department of Health, and the University of Alabama at Birmingham; the Human Research Protections Office at the University of Maryland, Baltimore approved secondary analysis of the data.

Measures

Incident PID

Clinical signs of PID, including cervical motion tenderness, uterine tenderness, and adnexal tenderness, were measured at each visit. For this analysis, PID was defined if at least 1 of these signs was present, based on current CDC guidelines for minimum clinical signs for the identification of presumptive PID [1]. Incident PID was the first visit at which the case definition was met after baseline. As this was a time-to-event data analysis, women not developing PID contributed data until their last study visit (right-censoring).

Bacterial Vaginosis

At each visit, vaginal fluid was collected for vaginal smears. Gram stains were performed and categorized using the Nugent scoring method as low, intermediate, or high (0–3, 4–6, and 7–10, respectively) [44]. High Nugent scores are indicative of BV and for clarity are termed "Nugent-BV" [17]. Additionally, clinical diagnosis of BV was assessed using Amsel criteria (having 3 of the following clinician-assessed signs: thin homogeneous vaginal discharge, vaginal pH >4.5, the presence of a fishy odor upon adding potassium hydroxide solution to vaginal secretions, and/or clue cells on microscopy) [45]. Diagnosis by Amsel criteria has also been recently termed "Amsel-BV" [17]. Clinicians directly questioned women regarding vaginal symptoms (including vaginal discharge, irritation, itching, burning, foul odor, and other), and then coded those that met Amsel criteria for BV as "symptomatic BV" or "asymptomatic BV" based on their clinical judgement [17]. Participants with symptomatic Amsel-BV were treated with standard of care (metronidazole or clindamycin [46]). To maintain temporal proximity with incident PID, and not concurrence, we evaluated the association between both Nugent-BV and Amsel-BV with PID at the subsequent visit 3 months later.

STI Screening

Endocervical swabs were utilized for all STI testing. As the study was conducted in the early 2000s, NG was assessed using Thayer-Martin agar culture and *Trichomonas vaginalis* (TV) was assessed by wet mount and InPouch culture at each visit [43]. Universal CT screening of participants at each visit was initiated partway into cohort recruitment based on introduction and new availability of ligase chain reaction (LCR; Abbott Laboratories, Abbott Park, Illinois). Prior to this, consent documents specifically informed women that CT screening was not done as part of routine study procedures, and indicated that participants should continue to follow up with their primary providers for routine CT screening [46]. All endocervical swab samples collected in the early part of the study, which had not previously been tested, were tested for CT with LCR after study completion. Because of this CT screening protocol in the first 2 years of study, there were some cases of untreated CT detected. CT, NG, and TV were defined as treated if the participant was prescribed or self-reported use of an unknown antibiotic or an antibiotic potentially active against that particular STI (ie, macrolides, tetracyclines or fluoroquinolones for CT, β-lactams, macrolides, tetracyclines or fluoroquinolones for

GC, and nitroimidazoles for TV), or a study clinician treated a participant for an STI in the 60-day interval prior to the visit, on the visit, or in the study interval after the visit. Detailed antibiotic use information was collected, including referral to STI clinics for treatment, clinic visit prescriptions, and those taken by participants for any STI and non-STI indications between study visits.

For analyses, we used both CT status at baseline that had not been treated with antibiotics (untreated CT) as well as CT (n = 28 untreated, n = 3 treated) antecedent to PID (at the visit prior to incident PID). NG and TV could not be modeled individually because there were very few untreated participants (n = 3 NG, n = 5 TV) who were observed to have incident PID. However, we conducted a sensitivity analysis incorporating a composite variable for both untreated CT/NG/TV at baseline and any CT/NG/TV antecedent to PID.

Time-Varying Survey Factors

Time-varying behaviors were queried at each visit and reflected history in the 3 months prior to each follow-up visit and 6 months prior to baseline. Frequency of douching was categorized in the prior interval to no douching, less than weekly, weekly, or more. Sexual risk behaviors included number of sexual partners (0–1, ≥2) and frequency of condom use (never, some of the time, always).

Sociodemographics

Sociodemographic factors including age (years), race (black, white, other), highest education level (high school and under, above high school), marital status (married, not married), and monthly household income (<$500, $500–$800, $800–$3000, >$3000) were queried at baseline. Due to very few participants in the "Other" racial group, results for this category were not reported.

Missing Data

There was <2% missing data for all measures except income and sexual behaviors, which had a maximum of 9% missingness. To address this, monotone multiple imputation was used to generate 10 imputed datasets that were used for all model analyses. We imputed household income using nonmissing data from race, age, education level, and marital status, as these were all associated with household income in our dataset. Missing sexual behaviors were imputed based on other sexual behaviors. For observations with all sexual behaviors missing (n = 3), age, education level, and marital status were used to impute missing sexual behaviors.

Statistical Analysis

We tested risk factors associated with incident PID including Nugent category, Amsel-BV (both symptomatic and asymptomatic), untreated CT infection at baseline, CT infection antecedent to PID, douching, sexual risk behaviors, and sociodemographic measures. We used χ^2 and Fisher exact tests for categorical factors, and Cochran–Armitage trend tests for ordinal factors.

Cox proportional hazards models were used to test whether Nugent score, Amsel-BV, untreated CT infection at baseline, CT infection antecedent to PID, douching, and sexual risk behaviors were associated with risk of incident PID. The time to incident PID was observed for women with PID diagnosis but right-censored at the last visit for those without incident PID. We generated both unadjusted models and models adjusted for all other factors. The proportional hazards assumption was assessed using scaled Schoenfeld residuals for all models. All analyses were conducted using SAS version 9.4 software (SAS Institute, Cary, North Carolina).

Analytic Sample

We excluded 123 participants who met any criteria of PID at baseline, 13 participants with missing PID data, and 434 participants with no follow-up visits. Observations with incomplete baseline measures after imputation (n = 92) were excluded. We removed 2 observations that were outliers based on high leverages. The final sample consisted of 2956 participants.

RESULTS

The 2956 participants contributed a total 2516.2 person-years, with a median of 349 days contributed per participant. The person-time contributed by each participant ranged from 49 to 456 days. Demographic and time-varying factors are displayed in Table 1 and Supplementary Table 1. Women in our analytic sample were predominantly black (82.6%) and unmarried (83.5%); most women had a high school education or less (73.4%) and had monthly income of either $500–$800 (29.5%) or $800–$3000 (42.3%). Of the 2956 participants, 160 (5.4%) had incident PID over the study period, equating to an incidence rate of 6.36 PID cases per 100 person-years. Sixteen women (10.0%) classified as having incident PID had all 3 clinical signs (cervical motion tenderness, uterine tenderness, and either right or left adnexal tenderness), and 34 women (21.3%) had 2 of these clinical signs. Thirty-one (19.4%) incident PID cases tested positive for CT at the visit prior to PID diagnosis. At the time of PID diagnosis, 12 (7.5%) cases tested positive for CT, 4 (2.5%) tested positive for NG, and 15 (9.4%) tested positive for TV. Nugent-BV was observed in 40.9% of the sample, while 8.2% of the sample had symptomatic Amsel-BV. Douching was reported by 40.0% of the sample. Furthermore, 66.2% of women with high Nugent scores at baseline had high Nugent scores prior to incident PID, whereas 65.0% of women with low Nugent scores at baseline had low Nugent scores prior to incident PID. Among women with intermediate BV at baseline, 36.0% had intermediate BV prior to incident PID. Overall, between baseline and visit prior to incident PID, 87.0% of women remained either within their same Nugent score category or an

Table 1. Bacterial Vaginosis, Chlamydia, and Covariates Across Incident Pelvic Inflammatory Disease (N = 2956)

Characteristic	Total (N = 2956), No.	No Incident PID (n = 2796 [94.6%]), %	Incident PID (n = 160 [5.4%]), %
Baseline demographic factors			
Age, y[a]			
18–20	710	24.3	20.0
21–24	959	32.3	34.4
25–29	579	19.5	21.3
30–34	328	11.2	9.4
≥35	380	12.7	15.0
Race[b]			
Black	2443	82.7	81.3
White	513	17.3	18.8
Education level[b]			
Greater than high school	788	26.8	25.0
High school or less	2168	73.3	75.0
Monthly income[a,c]			
Less than $500	566	20.4	28.5
$500–$800	803	29.8	24.3
$800–$3000	1152	42.5	38.9
Greater than $3000	200	7.3	8.3
Marital status[b]			
Unmarried	2471	**84.1**	**75.0**
Married	485	**15.9**	**25.0**
Time-varying factors[d]			
Douching[a,c]			
Never	1770	**60.6**	**49.4**
Less than weekly	947	**31.6**	**40.6**
Weekly or more	234	**7.8**	**10.0**
No. of sex partners[a,c]			
0	538	18.2	18.8
1	2256	76.7	72.5
≥2	157	5.1	8.8
Condom use[a,c]			
Never	1840	62.2	65.8
Some of the time	498	16.9	16.5
Always	610	20.9	17.7
BV and STIs			
Nugent score antecedent to PID[e]			
Low (0–3)	1114	**38.1**	**30.6**
Intermediate (4–6)	644	**21.8**	**21.9**
High (7–10)	1198	**40.1**	**47.5**
Amsel criteria antecedent to PID[e]			
No Amsel-BV	1770	**60.4**	**51.3**
Asymptomatic BV	1024	**34.4**	**39.4**
Symptomatic BV	162	**5.3**	**9.4**
Baseline untreated CT[f]			
No	2714	*92.0*	*88.1*
Yes	242	*8.0*	*11.9*
CT antecedent to PID[e,f]			
No	2234	75.3	80.6
Yes	722	24.7	19.4

Significant ($P < .05$) values are shown in bold; marginally significant variables (between $P < .05$ and $P < .10$) are shown in italics.

Abbreviations: BV, bacterial vaginosis; CT, *Chlamydia trachomatis*; PID, pelvic inflammatory disease; STI, sexually transmitted infection.

[a]Cochran–Armitage trend test.

[b]χ^2 test.

[c]Preimputation frequencies are reported for imputed variables.

[d]Baseline time-varying factors have a recall period of 6 months. All subsequent time points for time-varying factors have a recall period of 3 months.

[e]Antecedent to PID: at the visit prior to incident PID.

[f]Fisher exact test.

adjacent category of Nugent score (low to intermediate; intermediate to high). Supplementary Table 1 also shows patterns of BV and time-varying factors across time points.

Three (9.7%) CT cases diagnosed at the visit prior to incident PID were not treated. Four (12.9%) cases with a positive CT at the visit prior to incident PID had CT detected again at the time of PID diagnosis 3 months later. Those with incident PID were more likely to have untreated CT at baseline, engage in douching, and be married.

In univariate analysis (Table 2), both high Nugent score compared to low Nugent score (hazard ratio [HR], 1.42 [95% confidence interval {CI}, 1.01–2.02]) and symptomatic Amsel-BV compared to no Amsel-BV (HR, 2.04 [95% CI, 1.17–3.53]) were associated with greater risk of incident PID. After adjusting for sociodemographic and behavioral factors, as well as baseline untreated CT and CT antecedent to PID, asymptomatic Amsel-BV (adjusted HR [aHR], 1.52 [95% CI, 1.01–2.30]) and symptomatic Amsel-BV (aHR, 1.94 [95% CI, 1.11–3.41]) remained associated with greater risk of incident PID. Other factors associated with incident PID included baseline untreated CT, douching, and being married. Having CT antecedent to PID (90.3% of which was treated) was associated with lower risk of PID. The scaled Schoenfeld residual test did not indicate violations of proportional hazards ($P > .05$). Finally, when we conducted a sensitivity analysis using a combined NG/CT/TV variable in place of CT (for both baseline untreated and any antecedent CT), results remained consistent.

DISCUSSION

In a prospective observational study of 2956 women of reproductive age, we found that antecedent BV was associated with incident PID independent of relevant demographic, behavioral, and STI factors. Some, but not all, previous studies have found a positive association between BV and PID [21]. However, the majority of these studies have been cross-sectional, assessing BV at the time of PID diagnosis, and most did not control for

Table 2. Bacterial Vaginosis, Sexually Transmitted Infections, and Behaviors Associated With Incident Pelvic Inflammatory Disease (N = 2956)

Characteristic	Unadjusted	Adjusted[a]
Baseline demographic factors		
Age		
Continuous (years)	1.01 (.99–1.03)	1.00 (.97–1.02)
Race[a]		
Black	Reference	Reference
White	1.19 (.80–1.77)	0.96 (.60–1.55)
Education level[a]		
Greater than high school	Reference	Reference
High school or less	1.12 (.78–1.60)	1.05 (.70–1.58)
Monthly income		
Less than $500	Reference	Reference
$500–$800	**0.58 (.37–.91)**	**0.57 (.36–.90)**
$800–$3000	**0.65 (.43–.97)**	**0.57 (.37–.88)**
More than $3000	0.85 (.44–1.61)	0.82 (.42–1.60)
Marital status		
No	Reference	Reference
Yes	**1.83 (1.28–2.62)**	**2.15 (1.39–3.30)**
Time-varying factors, prior 3 mo[b]		
Vaginal douching		
Never	Reference	Reference
Less than weekly	**1.51 (1.09–2.10)**	**1.47 (1.03–2.09)**
Weekly or more	1.60 (.93–2.74)	1.42 (.75–2.28)
No. of sex partners		
0	Reference	Reference
1	**1.45 (1.05–2.00)**	0.87 (.55–1.37)
≥2	1.40 (.81–2.43)	1.65 (.81–3.35)
Condom use		
Never	Reference	Reference
Some of the time	0.95 (.62–1.46)	0.93 (.57–1.52)
Always	0.79 (.52–1.19)	0.86 (.53–1.40)
BV and STIs		
Nugent score antecedent to PID[c]		
Low (0–3)	Reference	Reference
Intermediate (4–6)	1.25 (.81–1.93)	1.24 (.77–2.01)
High (7–10)	**1.42 (1.01–2.02)**	**1.52 (1.01–2.30)**
Amsel criteria (3 out of 4) antecedent to PID[c]		
No Amsel-BV	Reference	Reference
Asymptomatic BV	*1.31 (.95–1.82)*	1.34 (.96–1.88)
Symptomatic BV	**2.04 (1.17–3.53)**	**1.94 (1.11–3.41)**
Untreated CT, baseline visit		
No	Reference	Reference
Yes	**1.55 (1.00–2.46)**	**3.87 (1.76–8.48)**
CT antecedent to PID[c]		
No	Reference	Reference
Yes	0.68 (.46–1.00)	**0.37 (.19–.72)**

Data are presented as hazard ratio (95% confidence interval). Significant ($P < .05$) values are shown in bold; marginally significant variables (between $P < .05$ and $P < .10$) are shown in italics.

Abbreviations: BV, bacterial vaginosis; CT, *Chlamydia trachomatis*; PID, pelvic inflammatory disease; STI, sexually transmitted infection.

[a]Amsel criteria and Nugent score were not adjusted for each other, but were adjusted for all other variables shown in table.

[b]Baseline time-varying factors have a recall period of 6 months. All subsequent time points for time-varying factors have a recall period of 3 months.

[c]Antecedent to PID: at the visit prior to incident PID.

infection with CT or NG [21]. The 1 large prospective study that followed women over 3 years did not show a relationship between Nugent-BV at baseline or 6 months prior to PID diagnosis, when controlling for NG or CT infection at entry into the study; 19.4% of women with BV in that study also had NG infection, CT infection, or both [28]. However, unlike our study, this report did not control for STI diagnosis at the time of BV diagnosis, antecedent to PID. Importantly, a subsequent analysis of the same data found that women with the highest tertile growth based on vaginal culture of several BV-associated organisms were more likely than those in the lowest tertile to experience incident PID [29]. A second study following women with PID prospectively after diagnosis found that specific BV-associated bacteria were more likely to be observed in women with endometritis as well as those with recurrent PID and infertility [27]. Finally, a small study (n = 17 women with PID, n = 17 without) utilizing quantitative polymerase chain reaction on vaginal specimens taken within 3 months prior to a PID diagnosis found that specific BV-associated bacteria (*Atopobium vaginae*, *Sneathia* species, BVAB-TM7, *Megasphaera* species, *Eggerthella*-like bacterium, and *Mobiluncus* species) were associated with incident PID, although they were unable to control for CT as all cases had CT and all controls did not [26]. Thus, our study expands the existing literature on the topic.

Additionally, we found that douching and having untreated CT at baseline were associated with incident PID, consistent with previous studies [15, 16]. Increased risk may relate to the potential for douching to enhance ascension of organisms from the lower to upper urogenital tract. It is unclear why marital status was associated with an increased risk of incident PID in this cohort when adjusting for biobehavioral risk factors. This association has been found in at least 1 other study [47], but that study did not control for behavioral factors.

Our study presents an analysis adjusted for major confounders, is prospective, and represents one of the largest cohorts to date in which the relationship between BV and PID has been examined. However, there are a number of limitations. First, the study is geographically and racially homogenous, as it was limited to clinics in the Birmingham, Alabama area, and was comprised primarily of black women. However, black women are disproportionately affected by BV and PID. The exclusion of participants not fluent in English is also an important limitation to consider. We were only able to follow participants for approximately 1 year, limiting our ability to detect incident PID beyond that timeframe. Future research examining the association between BV and PID longitudinally may help to better understand how BV affects long-term risk of PID. The PID definition using 1 or more clinical signs may lack specificity; however, the LSVF was designed around vaginitis as a research focus, and PID status was not recorded by clinicians. Even if it had been recorded, CDC guidelines at the time (1998) [46] were more stringent than current guidelines, requiring 3 clinical signs (lower abdominal tenderness, adnexal tenderness, and cervical motion tenderness for PID

diagnosis). The gold standard for PID diagnosis, histologic documentation, was not collected, though this necessitates invasive biopsy procedures and is impractical for standard clinical settings. We were also unable to test individual STIs other than CT due to infrequency among PID cases. Douching and sexual risk behaviors are self-reported, which may result in underascertainment. Finally, the vaginal microbiota may fluctuate [48], and assessing BV at 1 point in time may obscure relevant associations, although women with BV often have recurrent BV [49]. We assessed BV both by clinical criteria and microscopic assessment.

In summary, antecedent BV was significantly associated with incident PID in a large prospective cohort of women. New studies suggest that novel, more effective methods for vaginal microbiota optimization, including probiotics, may be on the horizon [42], raising interest in vaginal microbiota modification to prevent PID and other adverse reproductive outcomes. Further studies to elucidate the mechanisms by which BV, STIs, behaviors, and host responses interact to affect PID risk are needed.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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