MAJOR ARTICLE

Female Genital-Tract HIV Load Correlates Inversely with *Lactobacillus* Species but Positively with Bacterial Vaginosis and *Mycoplasma hominis*

Beverly E. Sha,[1] M. Reza Zariffard,[2] Qiong J. Wang,[3] Hua Y. Chen,[3] James Bremer,[2] Mardge H. Cohen,[4] and Gregory T. Spear[2]

[1]Department of Medicine, Section of Infectious Diseases, and [2]Department of Immunology/Microbiology, Rush University Medical Center, [3]Division of Epidemiology and Biostatistics, School of Public Health, University of Illinois at Chicago, and [4]Department of Medicine, CORE Center, Cook County Bureau of Health Sciences, Chicago, Illinois

Downloaded from https://academic.oup.com/jid/article/191/1/25/806112 by guest on 21 August 2022

Background. Bacterial vaginosis (BV) is associated with human immunodeficiency virus (HIV) acquisition. We examined the association between BV and BV-associated bacteria and expression of HIV in the female genital tract.

Methods. HIV RNA, lactobacilli, *Gardnerella vaginalis,* and *Mycoplasma hominis* in cervicovaginal lavage (CVL) samples were quantified by polymerase chain reaction (PCR). Gynecologic evaluation included Nugent score assessment, Amsel criteria assessment, detection of other genital-tract infections, and dysplasia grading. CD4 cell count, plasma HIV RNA level, and antiretroviral history were obtained.

Results. A total of 203 CVL samples from women with Nugent scores of 7–10 (BV group) and 203 samples from women with Nugent scores of 0–3 (no-BV group) were matched by plasma HIV RNA level and analyzed. After controlling for plasma HIV RNA level and Nugent score in univariate analyses, we found that *G. vaginalis* and *M. hominis* bacterial counts, *Candida* vaginitis, and herpes simplex virus (HSV) were positively associated with CVL HIV RNA levels. In multivariate analysis, only lactobacilli bacterial counts ($P = .006$; inverse association), *M. hominis* bacterial counts ($P = .0001$; positive association), *Candida* vaginitis ($P = .007$), and HSV ($P = .03$) were significantly associated with CVL HIV RNA levels.

Conclusion. Bacteria associated with BV increase genital-tract HIV RNA levels. Quantitative bacterial counts for lactobacilli and *M. hominis* are better correlates of CVL HIV RNA than are Nugent score or Amsel criteria. Since plasma virus and CD4 cell levels did not differ between the BV and no-BV groups, these data suggest that the bacterial flora associated with BV influence genital-tract HIV shedding.

In childbearing women, bacterial vaginosis (BV) is the most common cause of vaginitis. In BV, normal vaginal flora, consisting of hydrogen peroxide–producing lactobacilli, are replaced by *Gardnerella vaginalis, Mycoplasma hominis, Mobiluncus* species, and anaerobic gram-negative rods [1]. Earlier studies showed an association between BV and prevalence of HIV infection [2, 3]. More-recent prospective studies have demonstrated an association between BV and acquisition of HIV infection [4, 5]. These studies suggest the possibility that BV increases the risk of acquiring HIV through sexual intercourse.

The prevalence of BV in HIV-infected women and in HIV-uninfected women at high risk for HIV infection in the United States has been shown to be quite high in 2 large prospective cohorts, the HIV Epidemiology Research Study (HERS) and the Women's Interagency HIV Study (WIHS) [6–8]. BV, defined by Nugent Gram stain score, was present at baseline in 35% versus 33% (HERS) and 42% versus 48% (WIHS) of

Received 24 February 2004; accepted 12 July 2004; electronically published 2 December 2004.

Presented in part: 11th Conference on Retroviruses and Opportunistic Infections, San Francisco, 8–11 February 2004 (abstract 955).

Financial support: National Institutes of Health (grant P01 H040539); Women's Interagency HIV Study, which is funded by the National Institute of Allergy and Infectious Diseases, with supplemental funding from the National Cancer Institute, the National Institute on Drug Abuse, the National Institute of Dental and Craniofacial Research (grants U01-AI-35004, U01-AI-31834, U01-AI-34994, U01-AI-34989, U01-AI-34993, and U01-AI-42590), the National Institute of Child Health and Human Development (grant U01-HD-32632), and the National Center for Research Resources (grants MOI-RR-00071, MOI-RR-00079, and MOI-RR-00083).

Reprints or correspondence: Dr. Beverly E. Sha, 600 S. Paulina St., Ste. 143, Chicago, IL 60612 (bsha@rush.edu).

The Journal of Infectious Diseases 2005;191:25–32
© 2004 by the Infectious Diseases Society of America. All rights reserved.
0022-1899/2005/19101-0005$15.00

the HIV-infected and HIV-uninfected high-risk women, respectively. In the developing world, rates of BV are similarly high. Of 4718 women aged 15–59 years in Uganda, 50.9% were found to have BV by use of Nugent score [2]. In this cohort, the prevalence of HIV infection was 14.2% in the women with normal vaginal flora, compared with 21.3% in women with BV diagnosed by Nugent score.

Although BV has been associated with detection of HIV in the genital tract of HIV-infected women, the level of HIV in the cervicovaginal lavage (CVL) fluid was not quantified [9]. In Kenya, *Candida* vaginitis was associated with a 2-fold increased likelihood of detection of HIV-infected cells (as determined by the presence of HIV DNA) on vaginal swabs, but no effect of *Trichomonas* vaginitis or BV on prevalence of infected cells was identified [10].

Previously, we reported a polymerase chain reaction (PCR) method of quantifying several BV-related microorganisms, including *G. vaginalis, M. hominis,* and lactobacilli [11], in stored, frozen female-genital-tract (CVL) samples. We chose these specific bacteria because of their importance in BV [1, 12] and their potential interaction with HIV. Absence of lactobacilli has been associated with an increased risk of acquiring HIV infection [5, 13], and, in vitro, H_2O_2-producing *Lactobacillus acidophilus* has been shown to be viricidal to HIV [14]. In an earlier study, we demonstrated that *M. hominis* enhances HIV-1 transcription and replication mediated through activation of the κB enhancer [15] and that *G. vaginalis* lysates activate HIV expression in monocytoid and certain T cell lines [16]. Using Amsel criteria to define BV, we found that women with BV had a significantly higher number of *G. vaginalis* organisms ($P = .004$) and significantly lower numbers of lactobacilli organisms ($P = .013$) than did women without BV. No significant difference was seen in the number of *M. hominis* organisms between the 2 groups.

In the present study, we examined the association between BV and expression of HIV in the female genital tract by use of 3 different methods of detecting abnormal female-genital-tract flora (quantitative bacterial PCR, Nugent Gram stain score, and Amsel criteria [17, 18]), to explore the hypotheses that the presence of BV and loss of normal flora (lactobacilli) increases HIV expression in the female genital tract. An effect of bacteria on genital-tract HIV levels could help explain the association between BV and sexual transmission.

MATERIAL AND METHODS

Patient cohort. Four hundred six CVL samples obtained from HIV-infected women were selected from the WIHS repository. WIHS is a longitudinal, multicenter cohort study of HIV-infected and high-risk uninfected women followed at 6 clinical sites in the United States. After institutional review board approval was obtained, 2059 HIV-infected women were enrolled from October 1994 through November 1995. Informed consent was obtained from all subjects, and human-experimentation guidelines of the US Department of Health and Human Services and/or those of the investigators' institutions were followed in the conduct of clinical research. A detailed interview, physical and gynecologic examination, and laboratory monitoring are performed every 6 months [19].

Gynecologic examination included assessment for genital-tract infections and genital-tract dysplasia, as described elsewhere [7]. Women were scheduled for visits when they were not menstruating and were instructed to avoid inserting any substance into the genital tract for 48 h before their examination. Samples were collected in the following order: vaginal pH; vaginal swabs for trichomoniasis broth culture, utilizing a modified Diamond media (Remel), Gram stain, wet mount, and KOH with amine odor test; CVL collection; cervical swab for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* testing; and Pap smear. Lower genital-tract infections were diagnosed using the following criteria. Bacterial vaginosis was assessed by Amsel criteria [19] and Nugent score (central reading at the laboratory of Sharon Hillier, University of Pittsburgh–Magee Women's Hospital, Pittsburgh, PA [17]); *Candida* vaginitis was evaluated by clinician assessment of compatible symptoms and discharge, 10% KOH smear, and Pap smear; trichomoniasis was evaluated by clinician assessment, wet mount, culture, and Pap smear; gonorrhea was assessed by PACE-2 DNA probe (Genprobe); chlamydia was assessed by PACE-2 DNA probe (Genprobe) and chlamydia urine LCR (Abbott Laboratories); syphilis was determined by the presence of a chancre or positive *Treponema pallidum* direct fluorescent antibody (performed by Dr. Victoria Pope, Centers for Disease Control and Prevention, Atlanta, GA); active herpes simplex virus (HSV) was determined by the presence of typical vesicles or ulcers and by Pap smear; and human papillomavirus (HPV) was assessed by PCR, through use of methods published elsewhere [20]. For infections that could be diagnosed by use of more than one method, any positive result was considered to indicate a true infection. For BV, *Candida* vaginitis, and trichomoniasis, each method of diagnosis was also analyzed separately. Genital-tract dysplasia was assessed by Pap smear (central reading was performed at Kyto Diagnostics, New City, NY) and colposcopy. Peripheral CD4 cell counts and percentages were obtained by standard flow-cytometric methods, and plasma HIV RNA level, antiretroviral history, menopausal status, and history of hormone-therapy use were also simultaneously obtained.

CVL collection and processing. CVL samples were collected by irrigation of the cervix with 10 mL of nonbacteriostatic sterile saline, followed by aspiration from the posterior fornix. CVL was held on ice until processing, which occurred within 6 h of collection. CVL was gently vortexed to evenly distribute cells before aliquoting and freezing at −70°C. Two hundred

Downloaded from https://academic.oup.com/jid/article/191/1/25/806112 by guest on 21 August 2022

three CVL samples from women with Nugent scores of 7–10 (BV group) and 203 CVL samples from women with Nugent scores of 0–3 (non-BV group) were selected for analysis. Because plasma HIV RNA levels influence genital HIV shedding [21], CVL samples were randomly chosen from women with plasma HIV RNA levels in the detectable range (⩾4000 copies/mL), to allow us to match plasma HIV RNA levels between the 2 groups. Paired samples from the 2 groups were each matched within 0.3 $\log_{10}$ and by site and visit number (visits 2–5).

RNA quantitative assays. HIV RNA in CVL samples was quantified using the Standard Roche Amplicor HIV-1 Monitor test (version 1.0; Roche Diagnostic Corporation), with a lower limit of detection of 400 copies/mL. The standard extraction method (performed with 200 μL of CVL) was used with no modifications. HIV RNA in plasma was quantified using the nucleic acid sequence-based amplification (NASBA) system (Organon Teknika), with a lower limit of detection of 4000 copies/mL.

Bacteria. *M. hominis* and *G. vaginalis* were both obtained from the American Type Culture Collection (Rockville, MD), and *Lactobacillus crispatus* was kindly provided by Dr. Lin Tao (University of Illinois at Chicago Dental School). Bacteria were grown as described elsewhere [11].

DNA isolation. DNA was isolated as described elsewhere [11]. In brief, bacteria from CVL samples were pelleted by centrifugation and treated with a lysis buffer containing lysosyme (Sigma) for 20 min at room temperature. Bacteria were further disrupted by addition of SDS and proteinase K (Sigma) for 30 min at 37°C. Lysates were mixed with phenol/chloroform (aMRasco) and centrifuged. DNA was precipitated by incubation with absolute ethanol, with glycogen (Roche) added as a carrier.

Real-time PCR amplification. Real-time quantitation of bacterial DNA was performed as described elsewhere [11]. In brief, Syber Green PCR Core Reagents (PE Biosystems) were used. DNA (50 ng) was mixed with $MgCl_2$, dNTPs, primers, UNG (Applied Biosystems), and *Taq* Gold polymerase (Applied Biosystems). Amplification was performed by use of an ABI prism 5700 Thermocycler (Applied Biosystems). The primers for *M. hominis* were F-RNAHI (5′-CAATGGCTAATGCCGGATACGC-3′) and R-RNAH2 (5′-GGTACCGTCAGTCTGCAAT-3′); those for *G. vaginalis* were F-GVI (5′-TTACTGGTGTATCACTGTAAGG-3′) and R-GV3 (5′-CCGTCACAGGCTGAACAGT-3′). The *Lactobacillus* primer sequences (F-LBF [5′-ATGGAAGAACACCAGTGGCG-3′] and R-LBR [5′- CAGCACTGAGAGGCGGAAAC-3′]) were designed to detect both *L. jensenii* and *L. crispatus,* the 2 most common *Lactobacillus* species in the female genital tract [22]. All 3 primer sets resulted in amplification of DNA from the appropriate type of bacteria but showed no cross reactivity to the other 2 organisms or to any of a panel of 16 common genital-tract bacteria [11]. In each thermocycler run, 6 standards, consisting of 10^3–10^7 copies of DNA from the appropriate bacterium, were included to generate a standard curve [11]. CVL sample DNA that resulted in values higher than the standard curve were diluted and rerun. The number of bacteria in CVL was calculated by multiplying the total DNA isolated from CVL samples by the number of bacteria in 50 ng of DNA.

Statistical methods. Logarithmic transformation was applied to CVL HIV RNA levels and quantitative bacterial PCR counts before the analyses. In the sampling design of this study, a CVL sample from a woman with a low Nugent score (0–3) and a CVL sample from a woman with a high Nugent score (7–10) were matched by their quantitative plasma HIV RNA levels (within 0.3 $\log_{10}$). The analysis took this feature of design into consideration by including both the Nugent score and the plasma HIV RNA level as covariates in both the univariate and the multivariate analyses. The multivariate analysis included only covariates that were significant at $\alpha = 0.10$ in the univariate analysis.

The undetectable CVL HIV RNA bounds were treated as left-censoring data and were included in the analyses. After adjusting for other covariates, the effects of the quantitative bacterial PCR counts on CVL HIV RNA were analyzed by linear regression models accounting for the left censoring (SAS PROC LIFEREG). Because the number of subjects contributing repeated measures was small, and because the repeated measures were separated, in general, by ⩾6 months, the possible correlations among repeated measures were ignored for simplicity. SAS 8.1 software was used for all of the statistical analyses.

RESULTS

Three hundred sixty-two women contributed 406 CVL samples (203 CVL samples came from women with Nugent scores of 7–10 [BV group], and 203 CVL samples came from women with Nugent scores of 0–3 [no-BV group]). Three hundred twenty-one women contributed a single CVL sample, 38 women contributed 2 CVL samples, and 3 women contributed 3 CVL samples. Repeat visits during which a subject donated a second or third CVL sample were separated from the previous visits by ⩾1 intervals of 6 months.

The racial distributions in the groups were as follows: 119 (66%) African American, 38 (21%) Hispanic, 17 (10%) white, and 5 (3%) "other" for the BV group; and 99 (54%) African American, 47 (26%) Hispanic, 33 (18%) white, and 4 (2%) "other" for the no-BV group. There were more African American and fewer white women in the BV group ($P = .04$). The median age was 37 years (range, 21–62 years) and was not different between the 2 groups. CVL samples were collected from 30 January 1995 through 23 April 1997. Samples were selected from women with detectable plasma HIV RNA levels (⩾4000 copies/mL), and the distribution of antiretroviral use at the time of CVL collection was as follows: no antiretroviral

Downloaded from https://academic.oup.com/jid/article/191/1/25/806112 by guest on 21 August 2022

therapy, $N = 276$; monotherapy, $N = 94$; combination therapy, $N = 35$; highly active antiretroviral therapy, $N = 0$; and missing information, $N = 1$. Of the 406 CVL samples, 34 were collected from women who had previously undergone hysterectomy, and 38 samples were collected from women in menopause. Only 30 of the 406 CVL samples were collected from women taking any hormone therapy during the prior 6 months. Other than race, there were no statistically significant differences in the prevalence of these characteristics between the BV and no-BV groups.

After 14 December 1995, assessment for interfering genital-tract substances was performed by interview and was available for 240 of the 406 specimens. During the 48 h prior to the gynecologic examination, 6 women reported use of a vaginal tampon, 15 women had used douche, 18 had used a vaginal medication, and 50 had had vaginal intercourse (39 with a condom). For 35 CVL specimens, the examiner reported the presence of visible blood. There were no statistically significant differences in the incidence of these genital-tract practices or findings between the BV and no-BV groups.

The samples in the BV and no-BV groups were selected to match the plasma RNA levels between the 2 groups. Median values of plasma and CVL HIV RNA levels, CD4 cell counts, and bacterial CVL counts of *G. vaginalis, M. hominis,* and lactobacilli for the BV group and the no-BV group are detailed in table 1. Overall, $\log_{10}$ bacterial counts for *G. vaginalis* and *M. hominis* were significantly higher—and lactobacilli counts were significantly lower—in the BV group, compared with the no-BV group.

Increasing levels of *G. vaginalis* and *M. hominis* were strongly associated with BV as assessed by Nugent score ($P < .0001$ for both bacteria) and by Amsel criteria ($P < .0001$ for both bacteria). Decreasing levels of lactobacilli were strongly associated with BV as assessed by Nugent score ($P < .0001$) and by Amsel criteria ($P = .03$). Nugent score and Amsel criteria were strongly correlated ($P < .0001$).

Of the 406 CVL samples, 148 were collected from women with at least 1 other genital-tract infection, 83 from the BV group and 65 from the no-BV group ($P = .06$). For the BV group, the other genital-tract infections identified, given in decreasing order of frequency, were *Candida* vaginitis ($N = 40$), trichomoniasis ($N = 40$), HSV ($N = 12$), cervicitis (gonorrhea or chlamydia)/pelvic inflammatory disease (PID) ($N = 6$), and syphilis ($N = 0$). Thirteen CVL samples were collected from women with 2 other genital-tract infections (9 with *Candida* vaginitis and a second process), and 1 was collected from a woman with 3 other genital-tract infections (*Candida* vaginitis, trichomoniasis, and cervicitis). For the no-BV group, the genital-tract infections identified, given in decreasing order of frequency, were *Candida* vaginitis ($N = 49$), trichomoniasis ($N = 12$), HSV ($N = 7$), cervicitis (gonorrhea or chlamydia)/PID ($N = 5$), and syphilis ($N = 0$). Eight CVL samples were collected from women with *Candida* vaginitis and a second genital-tract infection. The BV group had significantly more cases of trichomoniasis than did the no-BV group ($P < .0001$). Otherwise, there were no statistically significant differences in the occurrences of the other genital-tract infections between the BV and no-BV groups.

Genital-tract dysplasia was also analyzed. Of the 406 CVL samples, 295 were collected from women with evidence of HPV, as assessed by PCR, and/or evidence of dysplasia, as assessed by Pap smear or colposcopy (152 in the BV group and 143 in the no-BV group; $P = .3$). Two hundred sixty-three samples were collected from women with a positive HPV DNA PCR result (133 in the BV group and 130 in the no-BV group; $P = .8$). Of these, only 19 in the BV group and 22 in the no-BV group were PCR-positive for HPV16 and/or HPV18 ($P = .6$)

In univariate analyses, after controlling for plasma HIV RNA, we found Nugent score to be associated with increased CVL HIV RNA levels (table 2). For the other variables, in univariate analyses, after controlling for both plasma HIV RNA and Nugent score, we found $\log_{10}$ *G. vaginalis* count, $\log_{10}$ *M. hominis* count, and presence of another inflammatory genital-tract infection to be associated with increased CVL HIV RNA levels

Table 1. Mean and median plasma and genital-tract HIV RNA level, CD4 cell counts, and quantitative bacterial polymerase chain reaction counts for the bacterial vaginosis (BV) group and the no-BV group.

	BV group ($N = 203$)			No-BV group ($N = 203$)			
Variable	Mean ± SD	Median	Range	Mean ± SD	Median	Range	P[a]
$\log_{10}$ CVL HIV RNA level, $\log_{10}$ copies/mL[b]	3.15 ± 0.69	2.88	2.6–5.65	3.01 ± 0.62	2.6	2.6–5.25	.03
$\log_{10}$ plasma HIV RNA level, $\log_{10}$ copies/mL	4.64 ± 0.66	4.58	3.62–6.54	4.64 ± 0.66	4.57	3.65–6.52	1.0
CD4 cell count, cells/mm^3	344 ± 235	328	0–1272	329 ± 259	290	0–1787	.6
$\log_{10}$ *Gardnerella vaginalis* count, $\log_{10}$ count/mL CVL	7.60 ± 2.53	8.50	0–10.21	5.65 ± 2.29	6.01	0–9.82	<.0001
$\log_{10}$ *Mycoplasma hominis* count, $\log_{10}$ count/mL CVL	5.67 ± 1.62	5.69	0–8.69	3.80 ± 1.92	3.79	0–8.13	<.0001
$\log_{10}$ lactobacilli count, $\log_{10}$ count/mL CVL	8.17 ± 1.10	8.28	0–10.08	8.80 ± 1.37	8.69	0–12.11	<.0001

NOTE. CVL, cervicovaginal lavage.

[a] Two-sample *t* test.

[b] One hundred twenty-three samples (114 women) and 92 samples (90 women), in the BV group and no-BV group, respectively, had CVL HIV RNA >400 copies/mL.

Downloaded from https://academic.oup.com/jid/article/191/1/25/806112 by guest on 21 August 2022

Table 2. Univariate and multivariate regression models of associations with cervicovaginal lavage (CVL) HIV RNA levels, after controlling for Nugent score and plasma HIV RNA level.

	Univariate model		Multivariate model	
Variable	Regression coefficient (95% CI)[a]	*P*	Regression coefficient (95% CI)	*P*
Nugent score[b]	0.33 (0.11 to 0.56)	**.004**	−0.09 (−0.34 to 0.16)	0.5
Plasma HIV RNA level, $\log_{10}$ copies/mL	0.53 (0.36 to 0.70)	**<.0001**	0.49 (0.33 to 0.65)	**<.0001**
CD4 cell count, cells/mm^3	−0.0002 (−0.0008 to 0.0003)	.4		
CD4 percentage	0.002 (−0.01 to 0.01)	.8		
Current antiretroviral therapy				
None	0 (0)	NA		
Monotherapy	−0.08 (−0.35 to 0.19)	.6		
Combination therapy	−0.26 (−0.69 to 0.16)	.2		
$\log_{10}$ *Gardnerella vaginalis* count, $\log_{10}$ count/mL CVL	0.07 (0.02 to 0.12)	**.004**	0.04 (−0.01 to 0.09)	.1
$\log_{10}$ *Mycoplasma hominis* count, $\log_{10}$ count/mL CVL	0.14 (0.09 to 0.20)	**<.0001**	0.12 (0.06 to 0.19)	**.0001**
$\log_{10}$ lactobacillus count, $\log_{10}$ count/mL CVL	−0.08 (−0.17 to 0.008)	**.07**	−0.12 (−0.21 to −0.04)	**.006**
Amsel criteria	0.18 (−0.14 to 0.49)	.3		
Other genital-tract infection	0.42 (0.19 to 0.64)	**.0003**	0.33 (0.11 to 0.55)	**.003**
Candida vaginitis	0.38 (0.12 to 0.64)	**.004**	0.34 (0.09 to 0.59)[c]	**.007**[c]
Clinician assessment[d]	0.36 (0.07 to 0.65)	**.01**	0.30 (0.03 to 0.58)[c]	**.03**[c]
KOH	0.42 (0.13 to 0.71)	**.004**	0.39 (0.12 to 0.67)[c]	**.005**[c]
Pap smear	0.47 (0.12 to 0.83)	**.01**	0.38 (0.04 to 0.72)[c]	**.03**[c]
Trichomoniasis	0.15 (−0.19 to 0.48)	.4		
Clinician assessment[e]	0.22 (−0.32 to 0.76)	.4		
Wet mount	0.12 (−0.41 to 0.65)	.6		
Culture	0.28 (−0.24 to 0.8)	.3		
Pap smear	0.06 (−0.36 to 0.48)	.8		
HSV	0.49 (−0.004 to 0.99)	**.05**	0.52 (0.05 to 0.98)[c]	**.03**[c]
Cervicitis/PID	−0.13 (−0.83 to 0.57)	.7		
Genital-tract dysplasia	0.13 (−0.13 to 0.39)	.3		
Any HPV PCR positive	0.02 (−0.22 to 0.25)	.9		
HPV16/HPV18 PCR positive	−0.28 (−0.66 to 0.10)	.2		
Abnormal cytologic result	0.15 (−0.07 to 0.38)	.2		
Use of hormone therapy	−0.05 (−0.47 to 0.37)	.8		
In menopause	−0.16 (−0.57 to 0.25)	.4		
Posthysterectomy	−0.22 (−0.64 to 0.19)	.3		

NOTE. Statistically significant *P* values (<.05) are indicated in boldface type. CI, confidence interval; HPV, human papillomavirus; HSV, herpes simplex virus; PCR, polymerase chain reaction; PID, pelvic inflammatory disease.

[a] One unit of change in the measure of each variable results in a change in $\log_{10}$ CVL HIV RNA level that is of a magnitude equal to the regression coefficient. For example, for a continuous variable such as $\log_{10}$ *G. vaginalis* count, a 10-fold increase in *G. vaginalis* (or one unit increase in $\log_{10}$ *G. vaginalis* count), results, on average, in a $10^{0.07}$-fold increase in CVL HIV RNA level. For a dichotomous variable such as antiretroviral therapy, receiving combination therapy results in a $10^{0.26}$-fold decrease, on average, in CVL HIV RNA level, compared with not receiving combination therapy.

[b] In univariate analysis of Nugent score, only plasma HIV RNA level was controlled for.

[c] CIs and *P* values for these variables were obtained by replacing the "Other genital-tract infection" variable in the multivariate model with each of the specific infection variables, one at a time. Only those specific infection variables that have significant effects are reported.

[d] Four cases were diagnosed by clinical assessment without confirmation by KOH smear or Pap smear findings.

[e] All cases diagnosed by clinical assessment were confirmed by wet mount, culture, or Pap smear findings.

(table 2). Although the racial distributions of the BV group and the no-BV group differed from one another, there was no association between race and HIV CVL RNA level ($P = .2$). In multivariate analyses, only $\log_{10}$ *M. hominis* count ($P = .0001$), $\log_{10}$ lactobacilli count ($P = .006$), and presence of another genital-tract infection ($P = .003$) were significantly associated with CVL HIV RNA level (table 2). In multivariate analyses, the specific other genital-tract infections found to be significantly associated with CVL HIV RNA levels were *Candida* vaginitis ($P = .007$) and HSV ($P = .03$). Trichomoniasis was not associated with CVL HIV RNA level. The conclusions regarding the effects of $\log_{10}$ *M. hominis* and $\log_{10}$ lactobacilli on genital-tract HIV RNA level were the same for the subgroup of subjects without another genital-tract infection.

DISCUSSION

BV is a common disorder in women throughout the world, regardless of HIV status. The diagnosis of BV by use of Amsel criteria is simple but relatively insensitive. In contrast, Nugent Gram stain scoring of vaginal secretions has a sensitivity of 93% and a specificity of 70% [17]. In our study, of the 203

Downloaded from https://academic.oup.com/jid/article/191/1/25/806112 by guest on 21 August 2022

samples that had BV diagnosed by Nugent score, only 75 (37%) met the definition of BV by Amsel criteria (data not shown). Vaginal cultures for *G. vaginalis* are sensitive but not specific, since 50%–60% of healthy asymptomatic women will be culture positive. Our data demonstrate that quantitative bacterial PCR for *G. vaginalis, M. hominis,* and lactobacilli is feasible and capable of quantifying numbers of BV-related bacteria in CVL samples. This PCR method significantly correlates with the Nugent Gram stain method in the diagnosis of BV. We found that women with BV diagnosed by Nugent score have significantly higher numbers of *G. vaginalis* ($P < .0001$) and *M. hominis* ($P < .0001$) and significantly lower numbers of lactobacilli ($P < .0001$) organisms in the CVL than do women without BV.

Although a PCR method of detecting BV is more labor intensive and costly than the Nugent Gram stain scoring method, it may offer several advantages in research settings. Unlike our PCR method, the Nugent Gram stain method cannot assess *M. hominis,* because of its lack of a cell wall. If uncentrifuged CVL samples are archived appropriately, quantitative PCR can be applied to samples even years after sample collection. Since PCR quantifies DNA rather than viable organisms, this technique could potentially be applied to stored samples that were not collected under conditions that would preserve organism viability.

Prior studies have demonstrated that the presence of BV is a risk factor for acquisition of HIV infection [4, 5]. We have confirmed data demonstrating that BV is significantly associated with the detection of HIV in the genital tract [9] and have now demonstrated that the actual level of HIV in the female genital tract is increased in the presence of BV. In univariate analyses, *G. vaginalis* count, *M. hominis* count, Nugent score, and presence of another lower genital-tract infection were associated with increased CVL HIV RNA level. Lactobacilli count was inversely associated with CVL HIV RNA level, at borderline significance ($P = .07$). Because we chose to select CVL samples by Nugent score, we had to control for Nugent score in our regression models. We were unable to detect an association between Amsel criteria and CVL HIV RNA level, possibly because Amsel is a less sensitive measure of detecting BV. In addition, our matching of the 2 groups by plasma HIV RNA level probably accounts for the lack of association between CVL HIV RNA level and CD4 cell count or antiretroviral therapy. In multivariate analyses, only *M. hominis* count, lactobacilli count, and presence of another lower genital-tract infection remained significantly associated with CVL HIV RNA level. Thus, in this study, quantitative bacterial PCR results correlated better with CVL HIV RNA level than did either Nugent score or Amsel criteria.

We were surprised to find that *G. vaginalis* bacterial counts were not associated with genital-tract HIV RNA level. Mean $\log_{10}$ bacterial counts for *G. vaginalis,* although higher in the BV group, were still quite high in the no-BV group (7.6 vs. 5.65, respectively). This high background prevalence of *G. vaginalis* may account for the lack of association with CVL HIV RNA level. In addition, since *M. hominis* and *G. vaginalis* counts are positively associated, we suspect that the effect of *G. vaginalis* was also obscured by the effect of *M. hominis* on genital-tract HIV RNA level. Nevertheless, our findings suggest that *M. hominis* has a more important effect on genital-tract HIV RNA level than does *G. vaginalis.*

Previously, we had shown that several bacteria associated with BV (*G. vaginalis, M. hominis, Peptostreptococcus asaccharolyticus, Prevotella bivia, Streptococcus agalactiae, Streptococcus constellatus,* and diphtheroid-like bacteria), isolated from the CVL of women with BV, have the ability to induce HIV expression in vitro [15, 16, 23, 24]. We also demonstrated that *M. hominis* and *G. vaginalis* activated HIV long-terminal repeat transcription in HIV-infected cells through an NF-κB–dependent mechanism [15, 16]. These findings could provide a mechanism that explains the association between *M. hominis* and HIV RNA in CVL. Others have shown that genital mycoplasmas stimulate tumor necrosis factor–α production from a murine macrophage cell line [25]. This may be another mechanism by which BV can up-regulate HIV expression. An alternate hypothesis, that increased plasma HIV load and/or level of immunosuppression influences the number of *M. hominis* and lactobacilli organisms in the genital tract, seems less plausible, since our BV group and no-BV group were matched by plasma HIV RNA level and had similar CD4 cell counts.

In our study, presence of another genital-tract infection also correlated with increasing CVL HIV RNA levels. Previously, viral shedding in cervicovaginal secretions has been shown to be significantly correlated with pregnancy, cervical ectopy, use of oral contraceptive pills, and cervicitis [10, 26, 27]. Specifically, *N. gonorrhoeae* and *C. trachomatis* have been shown to increase detection of HIV in the genital tract [26, 28]. Since so few women in our study had cervicitis at the time of CVL collection, we could not assess the effect of *N. gonorrhoeae* or *C. trachomatis* on genital-tract HIV RNA level. Enough women had *Candida* vaginitis and trichomoniasis that we could analyze the effects of these other causes of vaginitis separately. We were surprised to find that the presence of *Candida* vaginitis also had an independent effect on genital-tract HIV RNA level, since prior studies variably found an association between *Candida* vaginitis and increased HIV expression in the female genital tract [9, 10, 21]. Interestingly, an earlier study had demonstrated that treatment of *Candida* vulvovaginitis resulted in a 3.2-fold reduction in the concentration of cell-free virus in vaginal secretions and in an ~3-fold decrease in the likelihood of detecting HIV-infected cells [29]. Treatment of *Trichomonas* vaginitis resulted in a 4.2-fold reduction in the quantity of cell-free virus in vaginal secretions but in no decrease in shedding of

Downloaded from https://academic.oup.com/jid/article/191/1/25/806112 by guest on 21 August 2022

HIV-infected cells [29]. Treatment of BV did not result in a reduction of either cell-free HIV or HIV-infected cells [29]. We also saw no association between genital-tract HIV RNA level and trichomoniasis, cervical dysplasia, HPV shedding, or menopausal status. Despite the small numbers of women with HSV in our study, the association with HSV and CVL HIV RNA levels was significant ($P = .02$). An earlier cross-sectional study found a significant correlation between the quantities of HSV DNA and HIV RNA in cervical secretions of HSV-shedding women [30]. Unlike in several African studies, we did not find an association with genital-tract HIV shedding and use of hormone therapy [10, 26, 27]. However, since the number of women receiving hormone therapy was small, our study had limited power to detect an effect from hormonal contraceptives.

We treated repeated measures in a single subject as independent samples. Although this can result in underestimation of the variability, it does not introduce bias into estimates of regression coefficients. The underestimation of the variability can be expected to be small in our analysis, because repeated measures accounted for only 14% of the samples, and the repeated measures were separated by significant time intervals.

Evidence that disturbances in vaginal flora may lead to increased transmission of HIV from an HIV-infected woman to her sex partner has been inferential. H_2O_2-producing lactobacilli have been shown to have a viricidal effect on cell-free HIV and *N. gonorrhoeae* in vitro [14, 31]. Cultured bacteria (*G. vaginalis, M. hominis,* and *Streptococcus* species) from the CVL of HIV-infected women with BV have been shown to stimulate HIV expression in vitro [15, 16].

Our data demonstrate that the presence of BV, *Candida* vaginitis, and HSV in HIV-infected women with detectable plasma HIV RNA levels modestly increases the magnitude of genital-tract HIV shedding. Thus, sex partners of HIV-infected women with BV and *Candida* vaginitis may be at increased risk of acquiring HIV infection. Similarly, infants born to HIV-infected women with BV, *Candida* vaginitis, or genital-tract HSV disease may be at increased risk of acquiring HIV infection. A prior perinatal study showed that minimal elevations in CVL HIV RNA levels near the time of delivery significantly increased the risk of HIV transmission to the infant [32]. Treatment of active BV, *Candida* vaginitis, and HSV in HIV-infected women should be explored as a strategy to decrease the risk of HIV transmission to their sexual partners and to their infants during the delivery process.

Acknowledgments

We would like to thank all of the women who participated or are still participating in the Women's Interagency HIV Study. We gratefully acknowledge the support of the WIHS in providing samples and clinical data. The 6 clinical sites (principal investigators) are as follows: New York City/Bronx Consortium (Kathryn Anastos), Brooklyn, NY (Howard Minkoff), Washington DC Metropolitan Consortium (Mary Young), the Connie Wofsy Study Consortium of Northern California (Ruth Greenblatt and Herminia Palacio), Los Angeles County/Southern California Consortium (Alexandra Levine), and the Chicago Consortium (Mardge Cohen). We thank John O'Keefe of the University of Illinois at Chicago, for data management, and Cheryl Jennings of Rush University Medical Center, for cataloging the cervicovaginal lavage samples.

References

1. Sobel JD. Vaginitis. N Engl J Med **1997**; 337:1896–903.
2. Sewankambo N, Gray RH, Wawer MJ, et al. HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. Lancet **1997**; 350:546–50.
3. Kapiga SH, Sam NE, Shao JF, et al. HIV-1 epidemic among female bar and hotel workers in northern Tanzania: risk factors and opportunities for prevention. J Acquir Immune Defic Syndr **2002**; 29:409–17.
4. Taha TE, Hoover DR, Dallabetta GA, et al. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. AIDS **1998**; 12:1699–706.
5. Martin HL Jr, Richardson BA, Nyange PM, et al. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J Infect Dis **1999**; 180:1863–8.
6. Cu-Uvin S, Hogan JW, Warren D, et al. Prevalence of lower genital tract infections among human immunodeficiency virus (HIV)–seropositive and high-risk HIV-seronegative women. Clin Infect Dis **1999**; 29:1145–50.
7. Greenblatt RM, Baccetti P, Barkan S, et al. Lower genital tract infections among HIV-infected and high-risk uninfected women. Sex Transm Dis **1999**; 26:143–51.
8. Jamieson DJ, Duerr A, Klein RS, et al. Longitudinal analysis of bacterial vaginosis: findings from the HIV Epidemiology Research Study. Obstet Gynecol **2001**; 98:656–63.
9. Cu-Uvin S, Hogan JW, Caliendo AM, Harwell J, Mayer KH, Carpenter CJ, HIV Epidemiology Research Study. Association between bacterial vaginosis and expression of human immunodeficiency virus type 1 RNA in the female genital tract. Clin Infect Dis **2001**; 33:894–6.
10. Mostad SB, Overbaugh J, DeVange DM, et al. Hormonal contraception, vitamin A deficiency, and other risk factors for shedding of HIV-1 infected cells from the cervix and vagina. Lancet **1997**; 350:922–7.
11. Zariffard MR, Saifuddin M, Sha BE, Spear GT. Detection of bacterial vaginosis-related organisms by real-time PCR for lactobacilli, *Gardnerella vaginalis* and *Mycoplasma hominis.* FEMS Immunol Med Microbiol **2002**; 34:277–81.
12. Thorsen P, Jensen IP, Jeune B, Moller BR, Vestergaard BF. Few microorganisms associated with bacterial vaginosis may constitute the pathologic core: a population-based microbiologic study among 3596 pregnant women. Am J Obstet Gynecol **1998**; 178:580–7.
13. van de Wijgert JHHM, Mason PR, Gwanzura L, et al. Intravaginal practices, vaginal flora disturbances, and acquisition of sexually transmitted diseases in Zimbabwean women. J Infect Dis **2000**; 181:587–94.
14. Klebanoff SJ, Coombs RW. Virucidal effect of lactobacillus acidophilus on human immunodeficiency virus type 1: possible role in heterosexual transmission. J Exp Med **1991**; 174:289–92.
15. Al-Harthi L, Roebuck KA, Olinger GG, et al. Bacterial vaginosis-associated microflora isolated from the female genital tract activates HIV-1 expression. J Acquir Immune Defic Syndr **1999**; 21:194–202.
16. Hashemi FB, Ghassemi M, Roebuck KA, Spear GT. Activation of human immunodeficiency virus type 1 expression by *Gardnerella vaginalis.* J Infect Dis **1999**; 179:924–30.
17. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. J Clin Microbiol **1991**; 29:297–301.
18. Amsel R, Totten PA, Spiegel CA, Chen KCS, Eschenbach DA, Holmes KK. Nonspecific vaginitis: diagnostic criteria and epidemiologic associations. Am J Med **1983**; 74:14–22.
19. Barkan SE, Melnick SL, Martin-Preston S, et al. The Women's Inter-

Downloaded from https://academic.oup.com/jid/article/191/1/25/806112 by guest on 21 August 2022

agency HIV Study (WIHS)—design, methods, sample, cohort characteristics and comparison with reported AIDS cases in US women. Epidemiology **1998**; 9:117–25.
20. Palefsky JM, Minkoff H, Kalish LA, et al. Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women. J Natl Cancer Inst **1999**; 91:226–36.
21. Kovacs A, Wasserman SS, Burns D. et al. Determinants of HIV-1 shedding in the genital tract of women. Lancet **2001**; 358:1593–601.
22. Hillier SL. The vaginal microbial ecosystem and resistance to HIV. AIDS Res Hum Retroviruses **1998**; 14:S17–21.
23. Simoes JA, Hashemi FB, Aroutcheva AA, et al. Human immunodeficiency virus type 1 stimulatory activity by *Gardnerella vaginalis:* relationship to biotypes and other pathogenic characteristics. J Infect Dis **2001**; 184:22–7.
24. Hashemi FB, Ghassemi M, Faro, S, Aroutcheva A, Spear GT. Induction of human immunodeficiency virus type 1 expression by anaerobes associated with bacterial vaginosis. J Infect Dis **2000**; 181:1574–80.
25. Crouse DT, English K, Livingston L, Meals EA. Genital mycoplasmas stimulate tumor necrosis factor-α and inducible nitric oxide synthase production from a murine macrophage cell line. Pediatr Res **1998**; 44:785–90.
26. Mostad SB. Prevalence and correlates of HIV type 1 shedding in the female genital tract. AIDS Res Hum Retroviruses **1998**; 14(Suppl 1):S11–5.
27. Mostad SB, Kreiss JK. Shedding of HIV-1 in the genital tract. AIDS **1996**; 10:1305–15.
28. Ghys PD, Fransen K, Diallo MO, et al. The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Cote d'Ivoire. AIDS **1997**; 11:F85–93.
29. Wang CC, McClelland S, Reilly M, et al. The effect of treatment of vaginal infections on shedding of human immunodeficiency virus type 1. J Infect Dis **2001**; 183:1017–22.
30. McClelland RS, Wang CC, Overbaugh J, et al. Association between cervical shedding of herpes simplex virus and HIV-1. AIDS **2002**; 16:2425–30.
31. Zheng HS, Alcorn TM, Cohen MS. Effects of H_2O_2-producing lactobacilli on *Neisseria gonorrhoeae* growth and catalase activity. J Infect Dis **1994**; 170:1209–15.
32. Chuachoowong R, Shaffer N, Siriwasin W, et al. Short-course antenatal zidovudine reduces both cervicovaginal human immunodeficiency virus type 1 RNA levels and risk of perinatal transmission. J Infect Dis **2000**; 181:99–106.

Downloaded from https://academic.oup.com/jid/article/191/1/25/806112 by guest on 21 August 2022