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MAJOR ARTICLE

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Treatment Success Following Standard Antibiotic Treatment for Bacterial Vaginosis Is Not Associated With Pretreatment Genital Immune or Microbial Parameters

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Background. Bacterial vaginosis (BV) is a proinflammatory genital condition associated with adverse reproductive health outcomes, including increased HIV incidence. However, BV recurrence rates are high after standard antibiotic treatment. While the composition of the vaginal microbiota before BV treatment may be linked to BV recurrence, it is unclear whether the preceding genital immune milieu is predictive of treatment success.

Methods. Here we assessed whether baseline vaginal soluble immune factors or the composition of the vaginal microbiota predicted treatment success 1 month after metronidazole treatment in 2 separate cohorts of women with BV, 1 in the United States and 1 in Kenya; samples within 48 hours of BV treatment were also available for the US cohort.

Results. Neither soluble immune factors nor the composition of the vaginal microbiota before BV treatment was associated with treatment response in either cohort. In the US cohort, although the absolute abundances of key vaginal bacterial taxa pretreatment were not associated with treatment response, participants with sustained BV clearance had a more pronounced reduction in the absolute abundance of *Gardnerella vaginalis* immediately after treatment.

Conclusions. Pretreatment immune and microbial parameters were not predictive of BV treatment success in these clinical cohorts.

Keywords. HIV; antibiotics; bacterial vaginosis; immunology; mucosal immunology; vaginal microbiota.

Bacterial vaginosis (BV) is characterized by a vaginal microbiome that has a low abundance of *Lactobacillus* species and consists of a diverse array of gram-positive and -negative anaerobic bacteria [1]. Although BV is often asymptomatic, it causes genital inflammation, and for that reason it has been linked to a variety of adverse reproductive health outcomes, including elevated HIV acquisition risk [2]. The recommended treatment for symptomatic BV is a 5–7-day course of metronidazole or clindamycin [3]. However, BV recurrence rates approach two-thirds within a year of treatment [4], so defining pretreatment predictors of recurrence may permit the development of preemptive clinical strategies to maintain vaginal health. Elevated vaginal microbiota richness and evenness (ie, the total number and distribution of bacterial species in a population, respectively) before treatment have been linked with reduced odds of BV clearance [5], as has an increased ratio of vaginal *L. iners* to *G. vaginalis* before treatment [6]. Despite the profound mucosal immune impact of effective BV treatment [7, 8], baseline genital immune parameters that predict treatment success are not well defined. A recent study suggested that differences in vaginal chemokine levels and chemokine signaling before and during BV treatment may be associated with BV recurrence post-treatment [9].

Here, we hypothesized that elevated genital mucosal inflammation before standard antibiotic treatment for BV would be associated with lower rates of subsequent BV clearance. Our goal in this study was to analyze baseline data from 2 previously published clinical cohorts, 1 in the United States and 1 in Kenya, that assessed the vaginal immune impact of BV treatment to define microbial and immune predictors of treatment success.

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METHODS

Study Participants

Predictors of treatment outcome were assessed in 2 previously described clinical studies. Written informed consent was provided in both clinical studies before enrollment.

US-Based Cohort

Participants were recruited into a phase 2b, randomized, placebo-controlled clinical trial of *L. crispatus*–based live biotherapeutic LACTIN-V to reduce BV recurrence (NCT02766023) [10]. Briefly, 228 women with BV according to the US Food and Drug Administration definition (ie, Amsel criteria ≥3 and Nugent score ≥4) were given a 5-day course of topical metronidazole and then randomized to receive either LACTIN-V or matched placebo, which was topically applied daily for the following week and then twice weekly for 10 additional weeks. The present immune analyses included a randomly selected subset of 33 women who attended all clinic visits and were randomized to receive placebo after metronidazole treatment, described previously [11]. Vaginal swabs were collected immediately before intravaginal metronidazole treatment, within 48 hours of completing metronidazole, and 1 month postmetronidazole. BV was defined as a Nugent score ≥7. Immediately after collection, vaginal swabs were plunged into 2 mL of Starplex transport medium and frozen at −20°C or −80°C.

Kenya-Based Cohort

Participants with a Nugent score ≥7 were enrolled in a clinical study evaluating the genital immune impact of BV treatment [12]. Asymptomatic, sexually transmitted infection–free women with a Nugent score ≥7 were provided a 1-week course of oral metronidazole and returned 1 month after treatment initiation. Cervicovaginal secretions were collected at baseline and 1 month post-treatment using an Instead Softcup (Evofem, San Diego, CA, USA), which was vaginally inserted for 2 minutes, removed, stored on ice, and processed within 6 hours.

Soluble Immune Factor Measurements

In the US-based cohort, cervicovaginal fluid obtained from vaginal swabs was thawed and centrifuged at 2500×*g* for 30 minutes. Supernatant was then removed for immune factor analysis, and the remaining bacterial pellet was used for quantitative polymerase chain reaction (qPCR) analyses. The soluble immune factors interferon (IFN)-α2a, interleukin (IL)-17A, IL-1α, IL-6, IL-8, CXCL10, CCL4, CCL20, CXCL9, MMP-9, and sE-cad were measured in duplicate using the MSD multiplex chemiluminescent platform as previously described (Meso Scale Discovery) [7]. In the Kenya-based cohort, cervicovaginal fluid self-collected using the Instead Softcup was diluted 10-fold in phosphate-buffered saline (PBS) and stored at −80°C. Levels of IL-1α, IL-1β, IL-6, IL-8, IL-10, GM-CSF, IL-17, IFN-γ, tumor necrosis factor (TNF)–α, CCL3, CCL4, CCL20, CCL2, CCL5, and CXCL10 were measured in duplicate on the MSD platform (Meso Scale Discovery) [12].

DNA Extraction and qPCR

DNA was extracted from 175 µL of bacterial pellet from vaginal swab samples obtained in the US-based cohort using the Qiagen DNeasy PowerSoil kit according to the manufacturer's instructions. Targeted qPCR was used to estimate total bacterial load with a qPCR assay that targeted the 16-second rRNA gene and the absolute abundances of key bacterial species, including the 4 most common vaginal *Lactobacillus* spp. (*L. crispatus, L. iners, L. jensenii,* and *L. gasseri*) and 4 most common BV-associated bacterial taxa (*G. vaginalis, A. vaginae, Megasphaera* spp., and *Prevotella* spp.). All qPCR assays were TaqMan-based and performed on the QuantStudio 6 Flex Real-Time PCR System (Thermo Fisher Scientific). The protocol for quantification of *L. crispatus, L. iners, L. jensenii,* and *L. gasseri* absolute abundances with multiplex qPCR was adopted from Balashov and colleagues [13]. Absolute abundances of *G. vaginalis, A. vaginae*, and *Megasphaera* spp. were quantified with multiplex qPCR as per Kusters [14]. Total *Prevotella* absolute abundance was quantified with qPCR adopted from Martin [15]. Primer and probe sequences are presented in Supplementary Table 1. The total reaction volume for assays was 10 µL. Assays for *Prevotella* spp., *L. crispatus, L. iners, L. jensenii*, and *L. gasseri* were performed at 95°C for 10 minutes, 45 cycles at 95°C for 15 seconds, and then 60°C for 1 minute. Assays for *G. vaginalis, A. vaginae,* and *Megasphaera* spp. were performed at 95°C for 10 minutes, 45 cycles at 95°C for 15 seconds, and then 55°C for 1 minute. Data analysis was performed with QuantStudio Real-Time PCR Software, version 1.3 (Applied Biosystems). Copy numbers were quantified using the following equation, where ΔCt represents the difference in cycle threshold between a sample and negative control: Copy number = $2^{\Delta Ct}$.

DNA Extraction and Metagenomic Sequencing

One milliliter of each sample from the US-based cohort was plated from swab collection tubes into ZymoBIOMICS lysis solution for DNA extraction. DNA was extracted and processed with high-throughput automation liquid handlers (Agilent Bravo system and Labcyte ECHO instrument) to maintain constancy in sample experimentation and decrease laboratory processing time. The ZymoBIOMICS 96 MagBead DNA kit was followed as instructed to extract DNA from swab samples, water controls, and storage medium controls. Illumina library preparation was performed using a miniaturized protocol of the NEBNext Ultra II FS DNA Library Prep kit for DNA for the Labcyte ECHO instrument [16]. More than 25 million paired-end 150-bp reads per patient sample were collected on an Illumina NovaSeq 6000 instrument. The

CZ ID platform was used to process raw sequencing reads and remove host reads [17]. The VIRGO bioinformatic pipeline was used to align processed reads with established databases to identify microbes [18]. Samples with >100 000 reads were included in the present analyses. All metagenomic data can be downloaded from the NCBI Sequence Read Archive under BioProject ID PRJNA784288.

DNA Extraction and 16S rRNA Gene Sequencing

DNA extraction from genital secretions (200 µL) collected by Instead Softcup (Evofem, San Diego, CA, USA) was performed using the MoBio Microbiome kit automated on the Hamilton STAR robotic platform after a bead-beating step on a Qiagen Tissue Lyser II (20 Hz for 20 minutes) in 96–deep well plates. A dual-barcode system with primers 338F and 806R was used to amplify the V3-V4 regions of the 16S rRNA gene [19]; subsequent sequencing was performed on an Illumina HiSeq 2500 instrument (Illumina, San Diego, CA, USA) using a 300-bp paired-end protocol at the Genomic Resource Center at the University of Maryland School of Medicine, Institute for Genome Sciences. Sequence reads were trimmed if the average phred quality score of 4 consecutive base pairs was below 15 and retained only if their length was >75% of their original length. Paired reads were assembled using FLASH [20]. De-multiplexing was performed in QIIME (version 1.8.0) by binning sequences with the same barcode followed by quality trimming [19]. Detection of de novo and reference-based chimera was conducted in UCHIME (version 5.1) using the greengenes database of 16S rRNA gene sequences (Aug. 2013 version) as reference. The processed 16S rRNA gene amplicon sequences were assigned to genera and species using speciatIT software (http://www.ravel-lab.net/speciateit).

Statistical Analysis

Sociodemographic and behavioral characteristics were compared between groups with the Mann-Whitney *U* test or Fisher exact test. The association between baseline levels of soluble immune factors or bacterial absolute abundances and a Nugent score of ≥7 at follow-up was determined with binary logistic regression. The Fisher exact test was used to compare the proportion of baseline vaginal community state types between groups. Shannon diversity was compared between groups with the Mann-Whitney *U* test. The overall composition of the vaginal microbiota was compared between groups by performing an analysis of similarity (ANOSIM) on Bray-Curtis dissimilarities.

Patient Consent

For the US-based cohort, written informed consent was obtained from all participants before enrollment in the LACTIN-V clinical trial to prevent BV recurrence (NCT02766023), approved by the institutional review boards at San Francisco General Hospital, Stroger Hospital of Cook County, University of California San Diego Antiviral Research Center, and Washington University Infectious Disease Clinical Research Unit according to the principles expressed in the Declaration of Helskinki. The immunology and microbiology substudies were reviewed and approved by the University of Toronto HIV Research Ethics Board. For the Kenya-based cohort, written informed consent was obtained from all participants before enrollment. The study was approved by Institutional Review Boards at Kenyatta National Hospital (Nairobi, Kenya) and the Universities of Manitoba and Toronto (Canada) and was conducted according to the principles expressed in the Declaration of Helsinki.

RESULTS

A total of 78 female participants were included in this study, 33 from the US-based cohort and 45 from the Kenya-based cohort. Treatment nonresponse, defined as a Nugent score of ≥7 1 month after treatment, was observed among 14 (42%) participants in the US-based cohort and 18 (40%) participants in the Kenya-based cohort. Sociodemographic and behavioral characteristics did not differ between treatment responders and nonresponders (Table 1).

We first compared pretreatment levels of soluble immune factors in each cohort between responders and nonresponders. Baseline levels of soluble immune factors did not differ significantly between responders and nonresponders in either cohort, although in the Kenya-based cohort there was a trend toward elevated IL-10 among nonresponders, and all analytes tended to favor nonresponders (Figure 1).

Next, we compared the composition of the vaginal microbiota between responders and nonresponders. In the US-based cohort, the absolute abundances of key vaginal bacteria and total bacterial load were measured with taxon-specific and 16S rRNA gene qPCR, respectively, before BV treatment and did not significantly differ based on treatment response at 1 month (Figure 2). In addition, neither the pretreatment Shannon diversity score nor the ratio of *Gardnerella vaginalis* to *L. iners* relative abundance obtained with metagenomic sequencing (n = 15) or 16S rRNA gene sequencing (n = 45) differed between responders and nonresponders (Figure 2). Exploratory analyses were performed to compare the pretreatment relative abundance of the top 30 most abundant bacterial taxa identified with metagenomic sequencing (US-based cohort) or 16S rRNA gene sequencing (Kenya-based cohort). We performed this comparison on each cohort independently, and again there were no significant differences between responders and nonresponders in either cohort after controlling for multiple comparisons (Supplementary Tables 2 and 3).

Next, within the US-based cohort, we evaluated whether the immediate (within 48 hours of the end of treatment)

Table 1. Sociodemographic Characteristics

Characteristic	US-Based Cohort Responders n = 14	US-Based Cohort Nonresponders n = 19	US-Based Cohort *P* Value	Kenya-Based Cohort Responders n = 18	Kenya-Based Cohort Nonresponders n = 27	Kenya-Based Cohort *P* Value
Age, y	37 (20–44)	32 (21–44)	.2284	24.5 (19–38)	28 (20–46)	.08805
Race						
Asian	1 (7)	1 (5)	1	0 (0)	0 (0)	1
Black or African American	6 (43)	8 (42)	1	18 (100)	27 (100)	1
Multiracial	1 (7)	1 (5)	1	0 (0)	0 (0)	1
Unknown	1 (7)	2 (11)	1	0 (0)	0 (0)	1
White	5 (36)	7 (37)	1	0 (0)	0 (0)	1
Nugent score (pretreatment)	8 (5–10)	8 (6–10)	.5212	8.25 (2–10)	8 (0–9.5)	.225
Hormonal contraceptive use	5 (36)	4 (21)	.5897	16 (89)	20 (74)	.4027
Sexual intercourse within 3 d of starting metronidazole treatment	2 (14)	9 (47)	.1055	2 (11)	7 (26)	.4027

Data are presented as No. (%) or median (IQR). *P* values were generated with the Pearson chi-square test for categorical variables and Mann-Whitney *U* test for continuous variables.
Abbreviation: IQR, interquartile range.

impact of BV treatment on genital immunology and/or on the vaginal microbiota was able to predict treatment success at 1 month post-treatment; samples were not collected at this time point in the Kenya-based cohort. Among treatment responders, there was a trend to a greater reduction in vaginal MMP-9 ($P = .0523$) and IFN-α2a ($P = .0523$) concentrations immediately after metronidazole treatment (Figure 3), and the drop in *G. vaginalis* absolute abundance during treatment was more pronounced ($P = .0218$). In contrast, responders and nonresponders had comparable reductions in the absolute abundance of other BV-associated bacteria and little change in the absolute abundance of key *Lactobacillus* species immediately after metronidazole. Responders and nonresponders also had comparable reductions in overall bacterial load and Shannon diversity immediately after metronidazole (Figure 4).

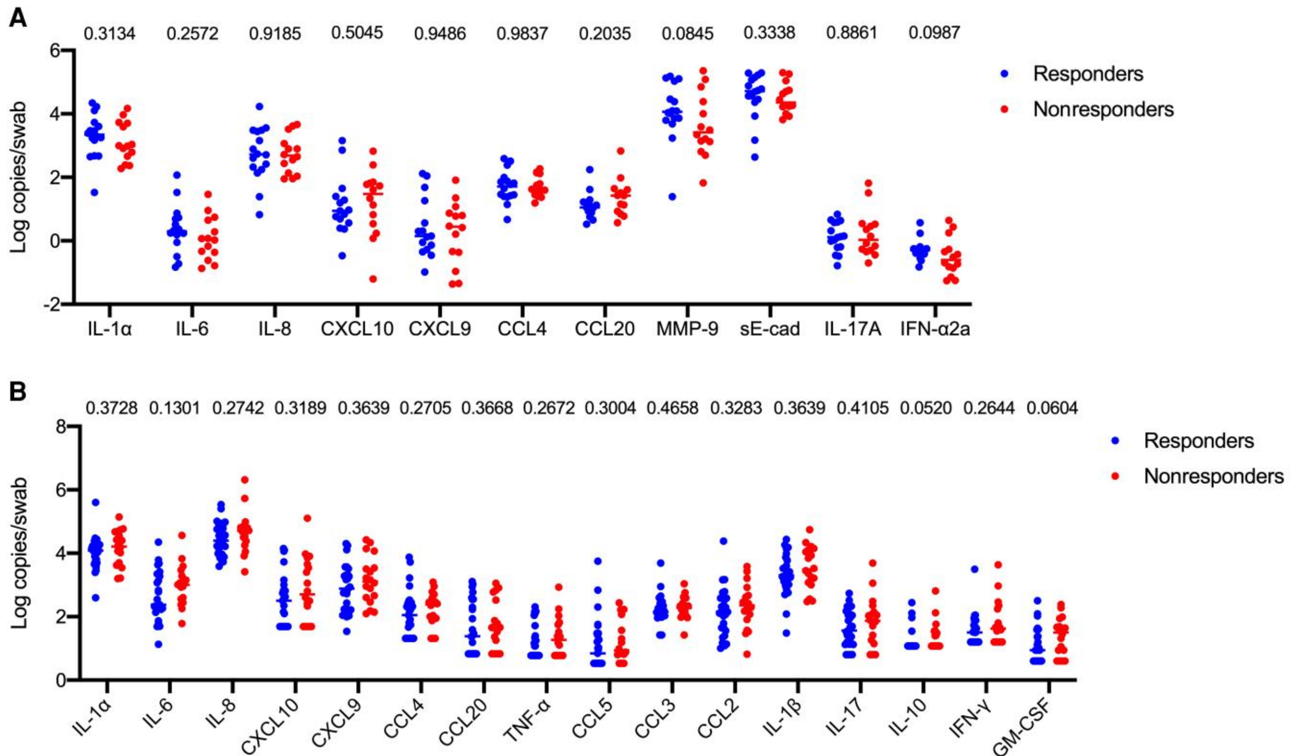

Figure 1. Association between pretreatment genital immune milieu and treatment response. Comparison of pretreatment levels of soluble immune factors between responders and nonresponders in the (*A*) US-based and (*B*) Kenya-based cohorts. Statistical tests were performed with the Mann-Whitney *U* test.

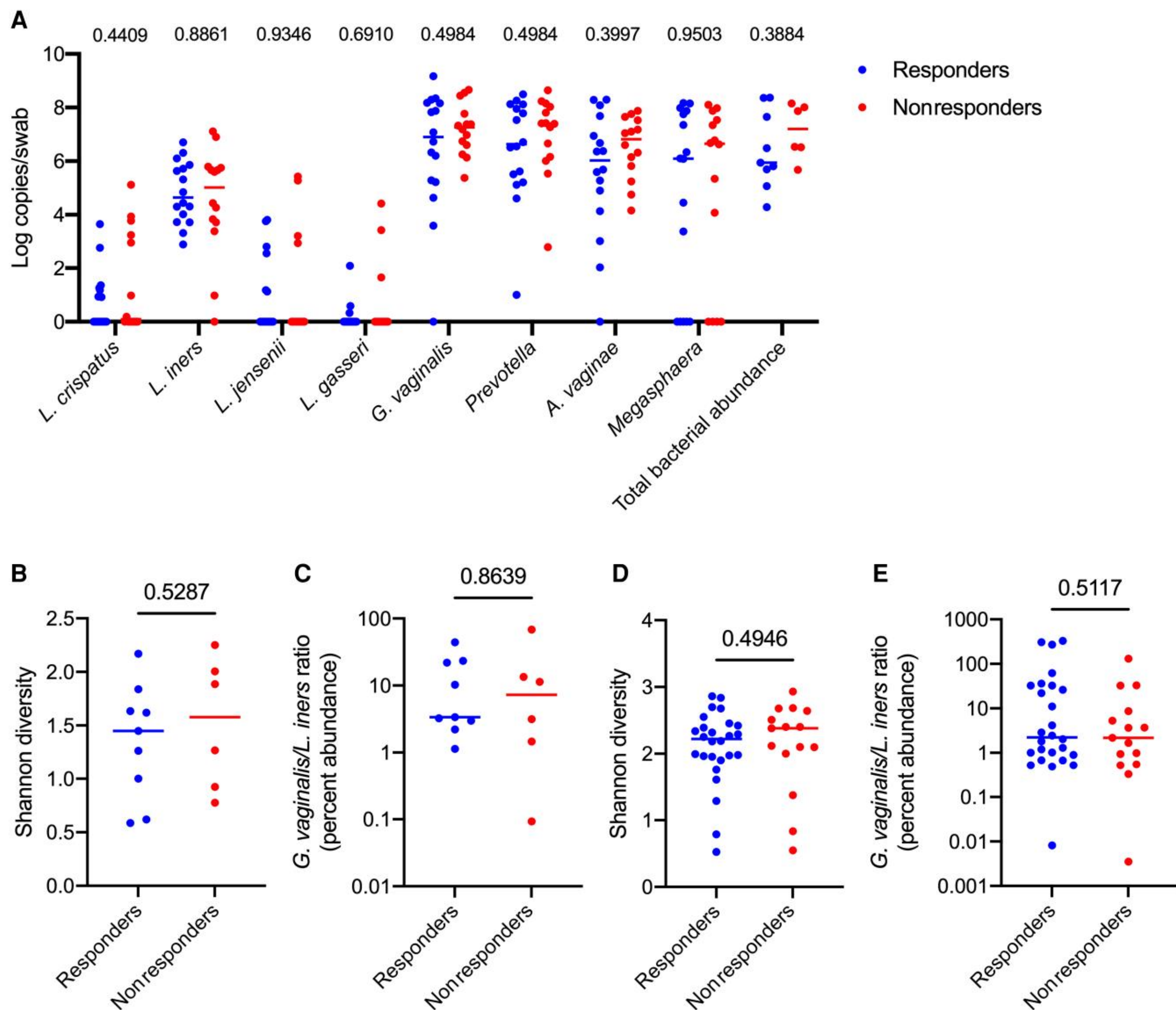

Figure 2. Composition of the vaginal microbiota before BV treatment based on treatment response. *A*, Difference in pretreatment absolute abundance of *L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri*, *G. vaginalis*, *Prevotella* spp., *A. vaginae*, *Megasphaera* spp., and total bacterial load (16S qPCR) between responders and nonresponders in the US-based cohort. Comparison of Shannon diversity of the vaginal microbiota before BV treatment in the (*B*) US-based and (*D*) Kenya-based cohorts. Comparison of the ratio of the pretreatment relative abundance of *G. vaginalis*:*L. iners* in the (*C*) US-based and (*E*) Kenya-based cohorts. Statistical tests were performed with the Mann-Whitney *U* test. Abbreviation: BV, bacterial vaginosis.

DISCUSSION

Compared with women with a *Lactobacillus*-predominant vaginal microbiota, prevalent BV is associated with profound differences in cervicovaginal immunology and (by definition) the cervicovaginal microbiota. Antibiotic treatment of BV is hampered by high treatment failure and recurrence rates, and it would be clinically useful to define pretreatment genital immune or microbial predictors of treatment success. Vaginal microbiota diversity has been closely linked to genital inflammation, and in 1 study it was associated with failure to clear BV after standard antibiotic treatment [5]. Thus, we hypothesized that women with a Nugent score ≥7 1 month after standard antibiotic treatment (ie, treatment nonresponders) would have elevated genital inflammation (represented by vaginal levels of IL-1α, a prototypical proinflammatory cytokine) and/or alterations in the vaginal microbiota before treatment. To test this hypothesis, we evaluated whether baseline differences in vaginal soluble immune factors or the composition of the vaginal microbiota could predict BV treatment response 1 month after metronidazole treatment in clinical cohorts of women with BV from the United States and Kenya. However, we observed no difference in pretreatment levels of prototypical proinflammatory cytokines (eg, IL-1α) or other soluble immune factors between treatment responders and nonresponders in either clinical cohort.

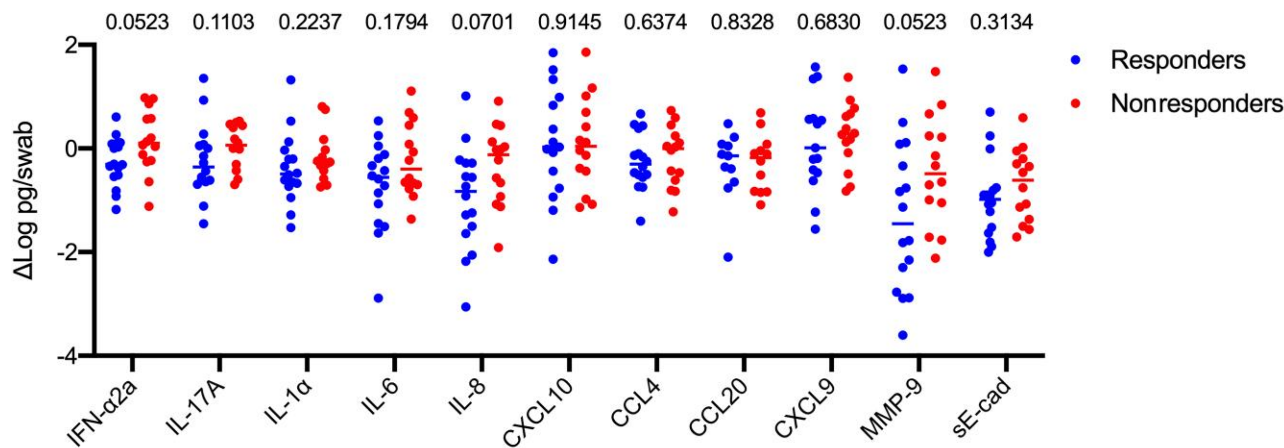

Figure 3. Change in soluble immune factors immediately after BV treatment based on treatment response at 1 month post-treatment. Comparison of the change in IFN-a2a, IL-17A, IL-1a, IL-6, IL-8, CXCL10, CCL4, CCL20, CXCL9, MMP-9, and sE-cad from baseline to 48 hours post-treatment between responders and nonresponders in the US-based cohort. Statistical tests were performed with the Mann-Whitney *U* test. Abbreviations: BV, bacterial vaginosis; IFN, interferon; IL, interleukin; CXCL, chemokine (C-X-C motif) ligand; CCL, chemokine (C-C motif) ligand; MMP, matrix metalloproteinase; sE-cad, soluble E-cadherin.

Despite the strong links between the vaginal microbiota and genital immunology, relatively few studies have explored the association between the genital immune milieu before BV treatment and subsequent treatment success. Elevated pretreatment vaginal levels of CXCL9 and differences in ectocervical cell chemokine signaling were reported among participants with a suboptimal response to metronidazole based on the Shannon diversity index, although differences in baseline levels of other soluble immune factors were not presented [9]. In the current study, we did not observe differences between treatment responders and nonresponders in pretreatment vaginal levels of CXCL9 or other soluble immune factors, although our study defined treatment success based on Nugent scores rather than based on Shannon diversity [9]. Interestingly, nonresponders tended to have elevated pretreatment levels of IL-10, an immunoregulatory cytokine. While IL-10 levels were only measured in the Kenyan cohort, this observation tends to contradict our initial hypothesis that responders would have elevated baseline frequencies of genital inflammation before BV treatment.

Many studies have explored the potential role of the vaginal microbiota in determining BV treatment outcome, with inconsistent results. Among women who have sex with women, pretreatment detection of a wide variety of BV-associated bacteria including BVAB1-3, *Peptoniphilus lacrimalis,* or *Megasphaera* spp. were associated with enhanced odds of BV recurrence post-treatment [21]. Interestingly, an increased pretreatment ratio of the relative abundance of *L. iners* to *G. vaginalis* [6], a reduced pretreatment relative abundance of *G. vaginalis* [22], and increased vaginal microbiota richness, evenness, and diversity [5] were linked to subsequent treatment failure in a study of women who have sex with men. In contrast to previous studies [5, 6], we did not observe differences in the pretreatment composition of the vaginal microbiota or the absolute abundance of key vaginal bacterial taxa based on treatment success, although in the US-based cohort the pretreatment relative abundance of *G. vaginalis* was higher among treatment responders. Unlike most previous studies, we were unable to evaluate predictors of BV recurrence per se because our treatment outcomes were within the US Food and Drug Administration window of cure for BV treatment [23]. However, we previously described high observed rates of molecular BV clearance immediately after BV treatment in the US-based cohort [7], suggesting that in this cohort treatment nonresponse at 1 month post-treatment likely reflected BV recurrence rather than a failure to eliminate BV-associated bacteria. In addition, treatment failure at 1 month was associated with a blunted reduction in the absolute abundance of *G. vaginalis* during BV treatment, suggesting that poor suppression of *G. vaginalis* after antibiotic treatment may be a key determinant of clinical response. Responders and nonresponders exhibited similar reductions in the absolute abundance of *Prevotella*, *A. vaginae,* and *Megasphaera* and total bacterial load immediately after metronidazole, suggesting that the *G. vaginalis* persistence observed among nonresponders reflected *G. vaginalis*–specific effects rather than poor clearance of BV-associated bacteria in general. Ngugi et al. evaluated bacterial dynamics over the course of BV treatment with metronidazole followed by topical administration of *L. crispatus* CTV-05 (LACTIN-V) based on subsequent colonization by *L. crispatus* CTV-05 and only observed a reduction in *G. vaginalis* absolute abundance (from the premetronidazole visit to the post-LACTIN-V visit) among women with CTV-05 colonization, but not women without CTV-05 colonization [24]. This study defined treatment success based on CTV-05 colonization (rather than Nugent BV clearance), but the results suggest that

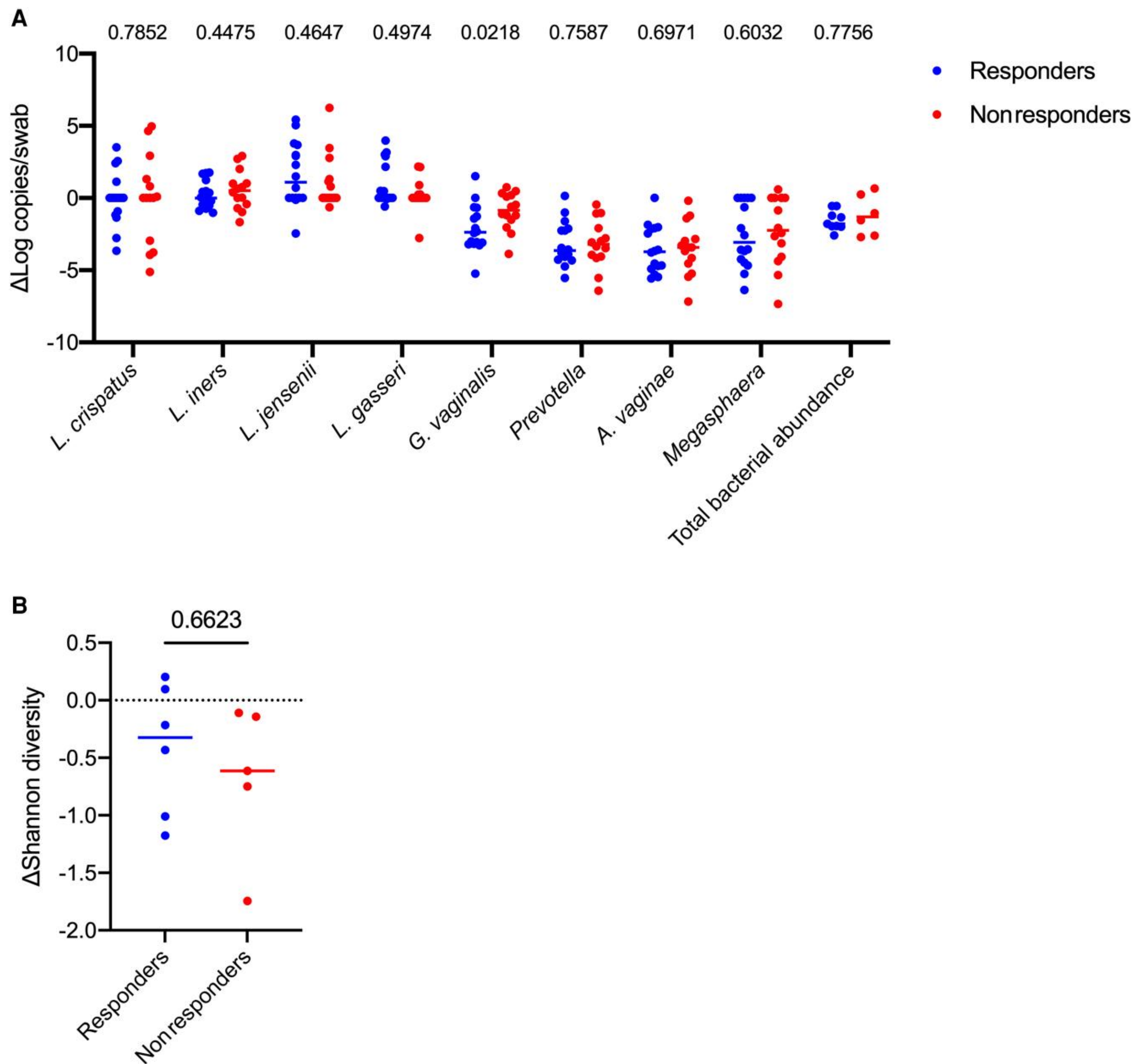

Figure 4. Short-term impact of BV treatment on the vaginal microbiota based on treatment response at 1 month post-treatment. Comparison of the change in (*A*) the absolute abundance of *L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri*, *G. vaginalis*, *Prevotella* spp., *A. vaginae*, *Megasphaera* spp., and total bacterial abundance and (*B*) Shannon diversity immediately after metronidazole treatment between responders and nonresponders in the US-based cohort. Statistical tests were performed with the Mann-Whitney *U* Test. Abbreviation: BV, bacterial vaginosis.

poor clearance of *G. vaginalis* may be a barrier to treatment success. *G. vaginalis* has been closely linked to biofilm formation, which has been implicated in failure to clear BV following standard antibiotics [25], but we were unable to evaluate the presence of biofilms.

Our study does have several limitations. The methodologies employed in both studies were not identical. Specifically, vaginal specimens were collected using different methods (swab vs Softcup), and different metronidazole regimens were used (oral vs intravaginal). However, there is no evidence that vaginal microbiome characterization differs based on sample collection method, and soluble immune factors are robustly detected using both methods [26]. Plasma samples were not available, which limited our ability to assess systemic immune parameters in our cohorts, but this was not our project focus. While different routes of metronidazole application were used in the 2 cohorts, as they had similar overall effects on the vaginal microbiota and immunology, this should, if anything, enhance the generalizability of our conclusions [27]. In addition, the specific immune factors analyzed in both cohorts differed

slightly, limiting our ability to confirm the association (or lack of association) between some factors and treatment response in both cohorts. The number of participants with available metagenomic data was relatively small in the US-based cohort, and the methodology for microbiome data generation differed in each cohort (metagenomic sequencing vs 16S rRNA gene sequencing), although the results were very similar to the 16S rRNA gene sequencing data from the slightly larger Kenya-based cohort. Generally, our cohort sizes were comparable to previous studies of BV treatment outcomes, with the exception of a study by Bradshaw and colleagues, which was substantially larger [4]. Ectocervical biopsies were not collected in either cohort, so the pretreatment ectocervical transcriptome associations described in a previous study could not be assessed [9]. We were unable to confirm our findings of *G. vaginalis* absolute abundance reduction and treatment success in the Kenya-based cohort, as no sample was collected immediately post-treatment; we were also unable to assess the association of vaginal biofilms with different *G. vaginalis* strains or treatment outcomes. Given the link between *G. vaginalis,* biofilm formation, and BV recurrence after treatment, future studies should explore these relationships in greater detail. Although sexual activity has been linked to BV recurrence [4], detailed sexual data were not collected in our cohorts. Finally, although adherence to metronidazole was not formally assessed in either cohort (eg, through directly observed treatment), high rates of BV clearance immediately after metronidazole treatment in the US-based cohort suggest high adherence, and the 1-month recurrence rates in our Kenya cohort [12] were consistent with previous studies [28, 29].

Overall, we did not find that baseline (pretreatment) genital immune or microbial parameters predicted BV treatment outcome, either in a US-based or Kenya-based cohort of women with BV. However, treatment success was associated with a more robust reduction in *G. vaginalis* abundance during metronidazole treatment, suggesting that antimicrobials specifically targeting *G. vaginalis* may enhance BV treatment success. Given the limited sample size available in the present analysis, future studies should explore these associations in larger cohorts.

Supplementary Data

Supplementary materials are available at *Open Forum 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.

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Potential conflicts of interest. C.R.C. has served as a paid consultant for Osel Inc. J.R. is co-founder of LUCA Biologics, a biotechnology company focusing on translating microbiome research into live biotherapeutics drugs for women's health. All other authors report no potential conflicts.

Author contributions. All authors contributed to the study. R.K., V.J., C.R.C., and A.H. contributed to study design. R.K., V.J., C.R.C., A.H., S.M., O.A., O.O., and K.S. contributed to study execution. E.A., V.J., and S.H. completed immune assays. E.A., B.C., J.R., M.K., E.C., and G.R.C. completed microbiome assays. E.A., R.K., and B.C. contributed to data analysis. E.A., R.K., C.R.C., A.H., and B.C. were involved in study interpretation. All authors had full access to the data used in the study, reviewed the manuscript, and had responsibility for the decision to submit for publication.

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