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Authors

Heffron, Renee
McClelland, R Scott
Balkus, Jennifer E
et al.

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Efficacy of Oral PrEP for HIV prevention among women with abnormal vaginal microbiota: a randomized, placebo controlled comparison

Renee Heffron, PhD,
Department of Global Health, University of Washington, Seattle, USA. Department of Epidemiology, University of Washington, Seattle, USA

Professor R. Scott McClelland, MD,
Department of Global Health, University of Washington, Seattle, USA. Department of Epidemiology, University of Washington, Seattle, USA. Department of Medicine, University of Washington, Seattle, USA

Jennifer E. Balkus, PhD,
Department of Epidemiology, University of Washington, Seattle, USA. Statistical Center for HIV/AIDS Research for Prevention, Fred Hutch, Seattle, USA

Professor Connie Celum, MD,
Department of Global Health, University of Washington, Seattle, USA. Department of Epidemiology, University of Washington, Seattle, USA. Department of Medicine, University of Washington, Seattle, USA

Professor Craig R. Cohen, MD,
Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California San Francisco, San Francisco, USA

Nelly Mugo, MBChB,
Department of Global Health, University of Washington, Seattle, USA. Centers for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya

Elizabeth Bukusi, MBChB,
Department of Global Health, University of Washington, Seattle, USA. Department of Obstetrics and Gynecology, University of Washington, Seattle, USA. Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya

Deborah Donnell, PhD,

Corresponding author: Renee Heffron, 325 Ninth Avenue Box 359927, Seattle, WA 98104, Tel: +1206 520 3817, Fax: +1 206 520 3831, rheffron@uw.edu.
*Team members are listed in the Acknowledgements

Conflicts of interest statement

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Author contributions

RH and JMB conceived the study. RH performed the statistical analyses and wrote the first draft of the manuscript. RSM and DF oversaw laboratory technicians that performed analyses of vaginal dysbiosis. All authors contributed critical revisions to the analysis and interpretation and reviewed the final manuscript draft.

Department of Global Health, University of Washington, Seattle, USA. Statistical Center for HIV/AIDS Research for Prevention, Fred Hutch, Seattle, USA. Vaccine and Infectious Disease Division, Fred Hutch, Seattle, USA

Professor Jairam Lingappa, MD,
Department of Global Health, University of Washington, Seattle, USA. Department of Medicine, University of Washington, Seattle, USA. Department of Pediatrics, University of Washington, Seattle, USA

James Kiarie, MBChB,
Human Reproduction Programme, World Health Organization, Geneva, Switzerland

Tina Fiedler, BS,
Vaccine and Infectious Disease Division, Fred Hutch, Seattle, USA

Matthew Munch, BS,
Vaccine and Infectious Disease Division, Fred Hutch, Seattle, USA

Professor David Fredricks, MD, and
Vaccine and Infectious Disease Division, Fred Hutch, Seattle, USA

Professor Jared M. Baeten, MD
Department of Global Health, University of Washington, Seattle, USA. Department of Epidemiology, University of Washington, Seattle, USA. Department of Medicine, University of Washington, Seattle, USA

for the Partners PrEP Study Team*

Summary

Background—Daily oral tenofovir-based pre-exposure prophylaxis (PrEP) demonstrated high efficacy for HIV prevention among women with high adherence but there is concern about the impact of abnormal vaginal microbiota on PrEP efficacy. The aim of this study was to investigate whether bacterial vaginosis modified the efficacy of oral PrEP.

Methods—Using prospectively collected data from women in the Partners PrEP Study, a placebo-controlled trial of daily oral PrEP conducted in Kenya and Uganda that had high efficacy in women, we assessed PrEP efficacy among subgroups of women defined by bacterial vaginosis (BV) status based on annually conducted microscopy and Nugent scoring (0–3 indicated normal, 4–6 intermediate, and 7–10 bacterial vaginosis) using Cox proportional hazards regression. In separate efficacy analyses, we also considered individual components of the score: detection of *Gardnerella vaginalis/Bacteroides* and non-detection of *Lactobacillus* as markers of abnormal microbiota.

Findings—Of 1470 women (median age=33 years), 357 (24%) had bacterial vaginosis at enrollment. In total, 45 women seroconverted to HIV. Using longitudinal data, PrEP had comparable HIV prevention efficacy among women with normal microbiota (PrEP arm HIV incidence: 0.6 per 100 person years, placebo arm HIV incidence: 2.5 per 100 person years, efficacy=77%), intermediate microbiota (PrEP arm HIV incidence: 1.8 per 100 person years, placebo arm HIV incidence: 3.5 per 100 person years, efficacy=63%), and BV (PrEP arm HIV incidence: 0.9 per 100 person years, placebo arm HIV incidence: 3.5 per 100 person years,

efficacy=73%) (interaction p-value=0.9). Similarly, oral PrEP efficacy was not different among women with detected versus undetected *Gardnerella vaginalis/Bacteroides* morphotypes (69% efficacy versus 77%, interaction p=0.7) and *Lactobacillus* morphotypes (70% versus 74%, interaction p=0.9).

Interpretation—Among African women with a high prevalence of BV and high PrEP adherence, the efficacy of daily oral PrEP for HIV prevention was not different among women with abnormal versus normal vaginal microbiota determined by Nugent score. These data are reassuring that oral PrEP delivery to women can continue without requiring concurrent testing for bacterial vaginosis or vaginal dysbiosis.

Keywords

PrEP; Africa; women; bacterial vaginosis; vaginal dysbiosis

Background

Pre-exposure prophylaxis (PrEP) with oral tenofovir-based pills is a highly effective HIV prevention strategy for women and men, with HIV protection exceeding 90% when adherence to the daily dosing regimen is high (1–3). In 2015, the World Health Organization recommended that PrEP be implemented as part of HIV prevention programs for people with substantial risk of HIV infection and multiple countries have now approved tenofovir-based PrEP for HIV prevention. Unlike condoms that require negotiation between partners for effective use, oral PrEP is discrete, offers the user personal control over HIV prevention, and provides empowerment and reduced anxiety to users (4). In addition to oral PrEP formulations, topical PrEP products such as a dapivirine-containing vaginal ring and 1% tenofovir gel have demonstrated moderate efficacy for prevention in some clinical trials, with higher efficacy correlated with evidence of greater adherence (5–8).

Two PrEP clinical trials failed to demonstrate HIV protection in women, in the context of low adherence, and pharmacokinetic data have shown that vaginal tissue concentrations of tenofovir reduce quickly with missed doses, resulting in suboptimal levels of tenofovir and impartial HIV protection (9–11). Consequently, biologic explanations for these findings have been proposed, including whether underlying conditions for women – inflammation, infection with sexually transmitted infections, bacterial vaginosis (BV), cervical ectopy, or exposure to a higher HIV inoculum – may potentially undermine the protective efficacy of PrEP (12, 13). The potential for these biologic mechanisms to affect PrEP efficacy may vary based on the topical or systemic delivery approach among different formulations. Recent data suggest that a non-*Lactobacillus* dominant vaginal microbiome may substantially reduce the prevention benefit of 1% tenofovir gel by increasing mucosal inflammation and HIV susceptibility and/or by reducing tenofovir metabolism (14, 15). No data have been reported thus far to understand whether vaginal dysbiosis could influence the HIV prevention efficacy of oral tenofovir-based PrEP.

In the Partners PrEP Study, an efficacy trial of daily oral PrEP among East African HIV serodiscordant couples, PrEP was efficacious for HIV prevention in both men and women, and in multiple high risk subgroups, including women aged <25 years and those whose HIV-

infected partners had a viral load >50,000 copies/ml (16). In a post-hoc analysis, we examined whether BV or microscopic evidence of vaginal dysbiosis on Gram stain was associated with lower oral PrEP efficacy compared to women with normal vaginal microbiota.

Methods

Study design

The Partners PrEP Study was a phase III, placebo-controlled, randomized trial of co-formulated emtricitabine/tenofovir disoproxil fumarate and single agent tenofovir disoproxil fumarate for HIV prevention among 4,747 HIV serodiscordant couples from 9 clinical research sites in Kenya and Uganda. Enrollment began on 3 July 2008. Eligible couples were randomized in a 1:1:1 fashion to emtricitabine/tenofovir disoproxil fumarate, tenofovir disoproxil fumarate, or placebo. Full procedures and results have been detailed previously (1). In the primary analysis, PrEP efficacy was 67% for tenofovir disoproxil fumarate and 75% for emtricitabine/tenofovir disoproxil fumarate (1). There was not a significant difference between the level of protection afforded by tenofovir disoproxil fumarate and emtricitabine/tenofovir disoproxil fumarate (17). Among women, tenofovir disoproxil fumarate efficacy was 71% (95% confidence interval [CI]: 37–87%) and emtricitabine/tenofovir disoproxil fumarate efficacy was 66% (95% CI: 28–84%) (1).

Participants

At enrollment, all participants were ≥18 years old and HIV-uninfected partners were not infected with hepatitis B virus and had normal renal function. HIV-uninfected women were encouraged to delay pregnancy until after their study involvement and study drug use. Standardized interviewer-administered questionnaires captured data on demographics at enrollment as well as sexual behavior, condom use, and contraceptive use at every visit.

Procedures

HIV-uninfected partners attended monthly study visits for HIV testing, prevention counseling, and to receive refills on study drug. Two HIV rapid tests were conducted in parallel at each visit. If at least one rapid test was positive, HIV EIA testing was conducted to confirm HIV seroconversion. At enrollment, annual visits, and when clinically indicated, genital exams were conducted and genital swab samples were collected from women for BV testing and screening for *Neisseria gonorrhoeae, Chlamydia trachomatis,* and *Trichomonas vaginalis*. For BV, vaginal swabs were rolled onto glass slides at the point of sample collection, air dried, and fixed with absolute methanol. At a single laboratory in Mombasa, Kenya, all slides were Gram stained and evaluated by microscopy for BV according to Nugent's criteria by two technologists with 20 years of experience each (18). For internal validity, both technologists read 10% of slides. For external quality assurance, a panel of slides was sent periodically to the Mombasa lab from the University of Washington. For *Neisseria gonorrhoeae, Chlamydia trachomatis,* and *Trichomonas vaginalis*, endocervical swabs were collected and tested with the GenProbe Aptima Combo2 (Hologic Inc, San Diego, California). In addition to diagnostic testing, STI symptoms were assessed quarterly and when clinically indicated. Women found to have any genital infection, syndromically or

diagnostically, were treated according to national guidelines. All laboratory testing and clinical management were conducted by staff blinded to PrEP versus placebo assignment. In a subset of 107 women from the placebo arm, we used results from taxon-specific quantitative polymerase chain reaction (qPCR) analysis assessing concentrations of *Gardnerella vaginalis* and *Lactobacillus crispatus* (19), to examine the relationship between the Gram stain results and the bacterial concentration of these key species.

The study protocol was approved by human subjects committees at the University of Washington and all study sites. All participants provided written informed consent in their preferred language. This analysis includes data collected prior to 10 July 2011, the time when the placebo arm of the study was stopped following recommendation from the study's independent data safety and monitoring board due to substantial protection being provided by both PrEP agents (1).

Statistical analysis

The primary exposure of interest was the interaction between PrEP and BV and the outcome for all models was incident HIV infection. Women randomized to tenofovir disoproxil fumarate and emtricitabine/tenofovir disoproxil fumarate were combined into one group since these medications were similarly effective in preventing HIV infection (17). BV was defined as having a Nugent score of 7–10. Women with scores of 0–3 were considered to have normal microbiota and 4–6 were considered to have intermediate microbiota.

The Nugent score is a weighted combination based on microscopic evaluation of three bacterial morphotypes: *Lactobacillus* (maximum score=4), *Gardnerella vaginalis* or *Bacteroides* combined (maximum score=4), and curved Gram-variable rods (maximum score=2) (18, 20, 21). The vaginal microbiota is considered optimal when *Lactobacillus* are the predominant bacterial morphotype and *Gardnerella*/*Bacteroides* are absent, and suboptimal when there is predominance by non-*Lactobacillus* morphotypes, such as *Gardnerella vaginalis*, on Gram stain. Intermediate vaginal microbiota and BV have been associated with increased risk of HIV acquisition in multiple studies (22). Thus, in separate analyses, we considered the interaction between PrEP and women's scores for the *Lactobacillus* and *Gardnerella vaginalis*/*Bacteroides* components of the Nugent score. For those analyses, scores of 0 from the individual component (e.g. the *Lactobacillus* component) were considered undetectable and scores of 1–4 were considered detected.

In Cox proportional hazards regression models, we estimated PrEP efficacy (PrEP vs. placebo, as randomized) among periods categorized by their BV (or *Lactobacillus* or *Gardnerella vaginalis/Bacteroides*) status. We included an interaction term to assess whether BV status modified PrEP efficacy, using the Wald test to calculate interaction p-values. BV status was a time-dependent variable, with one result carried forward until another result was available. Separately, we using Cox proportional hazards regression models with time-varying covariates to estimate the effect of having intermediate microbiota (Nugent 4–6) or BV (Nugent 7–10) on HIV incidence, stratified by study arm with *a priori* determined adjustment for age, STIs at enrollment, and time-varying unprotected sex and hormonal contraceptive use. In sensitivity analyses, we substituted baseline (instead of time-varying) BV, *Gardnerella vaginalis/Bacteroides*, and *Lactobacillus* status in separate efficacy models.

We compared the log10-transformed qPCR results across Gram stain categories using Wilcoxon test. Analyses were performed using SAS 9.4 (Cary, USA).

Role of the funding source

The funder(s) of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Findings

A total of 1,470 HIV-uninfected women with baseline Nugent scores were included for analysis, contributing a total of 2827 person-years (3.6% of person time was excluded due to missing baseline Nugent score). The median age was 33 years (interquartile range [IQR] 28–39), nearly all were married, and most had at least one child (median 3, IQR 1–5) (Table 1). Women had sex a median of 4 times (IQR 2–8) with their HIV-infected study partner in the month prior to enrollment and very few (0.5%) reported additional partners. Two-thirds were randomized to emtricitabine/tenofovir disoproxil fumarate or tenofovir disoproxil fumarate PrEP.

At enrollment, 24.3% (357/1470) of women had Nugent 7–10, 12.2% (180/1470) had Nugent 4–6, and 63.5% (933/1470) had Nugent scores of 0–3. Gram stain showing *Lactobacillus* morphotypes was present in 80% (1175/1469) of samples at baseline and Gram stain showing *Gardnerella vaginalis/Bacteroides* morphotypes was present in 37.2% (546/1469) of samples. Among samples from enrollment and follow up (median samples per woman=2, IQR 2–3) with Nugent scores 0–3, *Gardnerella vaginalis/Bacteroides* morphotypes were present in 1.8% (60/3272) of samples while *Gardnerella vaginalis/Bacteroides* morphotypes were present in 90.8% (540/595) and 100.0% (1079/1079) of samples with Nugent scores 4–6 or 7–10. Thus, in general, women tended to sort into groups with greater presence of *Lactobacillus* or *Gardnerella vaginalis/Bacteroides* with BV status highly correlated with this grouping. Results from qPCR data found that relative to women with Nugent scores 0–3, women with Nugent scores 4–6 and 7–10 had greater concentrations of *Gardnerella vaginalis* ($p<0.0001$ for both comparisons) and women with Nugent scores 7–10 had lower concentrations of *Lactobacillus crispatus* ($p=0.003$) (Figure 1). Examining Nugent scores longitudinally, 48.2% (573/1190) of women consistently scored 0–3, 16.1% (191/1190) consistently scored of 4–10, and 35.8% (426/1190) fluctuated during follow up.

At enrollment, 8% (117/1470) of women (11.5% [41/357] with BV, 16.1% [29/180] with intermediate microbiota, and 5.0% [47/933] with normal microbiota) were infected with *N. gonorrhoeae, C. trachomatis,* or *T. vaginalis*. Treatment with metronidazole or other medications recommended by CDC/WHO for vaginal symptoms common with BV was given to women at 8% (124/1470) of enrollment visits and 2% (222/9865) of quarterly follow-up visits.

There were a total of 45 incident HIV infections. The overall HIV incidence rate was 0.9 and 2.8 per 100 person-years among women randomized to PrEP and placebo, respectively,

yielding an HIV prevention efficacy from PrEP of 70.49% (95% CI 45.45–84.03%). Among longitudinal periods when women had Nugent scores of 0–3, 4–6, and 7–10, HIV incidence was 2.5, 3.5, and 3.5 per 100 person-years, respectively, in the placebo arm and 0.6 1.8, and 0.9, respectively, in the PrEP arm (Table 2, section A). Nugent scores 4–6 (adjusted HR 1.8, 95% CI 0.8–4.1, p=0.2) and 7–10 (adjusted HR 1.4, 95% CI 0.7–3.0, p=0.3) were associated with an increased risk for HIV acquisition relative to women with normal microbiota, although neither was statistically significant.

We observed comparable HIV protection efficacies among periods from women with Nugent scores 7–10 (72.50%, p=0.040), 4–6 (62.72%, p=0.196), and 0–3 (76.55%, p=0.001) (interaction p=0.871, Figure 2). Efficacy estimates were very similar after adjusting for age, STIs at enrollment, and hormonal contraceptive use (70.56% for Nugent 7–10, 64.23% for Nugent 4–6, and 83.77% for Nugent 0–3). Additional adjustment for receiving treatment for BV symptoms within the past 3 months did not alter the relationship between Nugent category and HIV incidence.

Overall results were similar when looking at the *Gardnerella vaginalis*/*Bacteroides* and *Lactobacillus* components of the Nugent score as separate markers of vaginal dysbiosis. PrEP efficacy was 68.62% and 76.72% among women with detectable and undetectable *Gardnerella vaginalis*/*Bacteroides* by Gram stain (interaction p-value=0.65) and 70.48% and 74.08% among women with detectable and undetectable *Lactobacillus* (interaction p-value 0.9).

When women were categorized based on their Nugent score at baseline, rather than in a time-dependent fashion over follow-up, PrEP efficacy comparisons were similar within categories defined by the full Nugent score as well as the *Gardnerella vaginosis*/*Bacteroides* and *Lactobacillus* components of the score (Table 2, section B).

Interpretation

We observed no indication that the protective benefit of daily oral PrEP was reduced in East African women with Gram stain evidence of BV or vaginal dysbiosis. BV was common: 25% of the women in this cohort entered the study with BV by Gram stain criteria, 37% had *Gardnerella vaginalis*/*Bacteroides* morphotypes detected. Gram stain results were consistent with qPCR testing in a subset of women, showing that women tended to sort into groups with greater presence of either *Lactobacillus* or *Gardnerella* species. Similar to other studies, abnormal vaginal microbiota appeared to be associated with increased risk of HIV acquisition, highlighting the important need for oral PrEP to work in settings where vaginal dysbiosis is common (23). Our data are reassuring that oral PrEP is efficacious for women with abnormal vaginal microbiota.

In CAPRISA 004, a randomized trial of 1% tenofovir gel for HIV prevention among high risk South African women, primary results demonstrated moderate protective benefit of the gel (39% efficacy, 95% CI: 6–60%)(5). Recent data suggest that vaginal dysbiosis, as measured using metaproteomic methods, may moderate the protective effect of the gel: women with non-*Lactobacillus* dominant microbiota received no protective benefit from the

gel, whereas women with *Lactobacillus*-dominant microbiota saw a protective benefit (15). We used a different method to evaluate vaginal dysbiosis (Gram stain, supported by qPCR testing in a subset), although it is likely that the approaches used in the CAPRISA 004 analysis and in our testing would have generally classified women's dysbiosis status similarly. Our results do not show the striking difference for orally-delivered tenofovir-based PrEP as seen in the CAPRISA 004 report, where tenofovir was delivered topically.

The metabolic processes for oral PrEP and tenofovir gel are different. The active agents in oral PrEP are systemically distributed in order to be present in mucosal surfaces and vaginal tissues (24, 25). In contrast, 1% tenofovir gel is at greatest concentrations in the vagina and penetrates only minimally beyond the mucosa and into plasma (26, 27). Thus the pathways that oral and topical formulations take to reach HIV target cells and prevent HIV acquisition are distinct. Since oral PrEP is absorbed and metabolized systemically, it is less plausible that a local mediator, such as BV or vaginal dysbiosis, could modulate the protective benefit of oral PrEP, compared to the potential effects that a local mediator might have on topically-delivered PrEP agents.

Adherence to the daily oral PrEP regimen was very high in this cohort, with prior analyses demonstrating that >80% of participants had plasma levels consistent with daily use (1). Recent work to understand the pharmacokinetics and pharmacodynamics of tenofovir-based PrEP have suggested that the daily dosing regimen may be more forgiving among men who have sex with men than heterosexual women when single doses are missed, based on tenofovir metabolism in cervicovaginal versus rectal tissue (9). Full understanding of the role of the genital microbiome in potentially modifying this metabolism and the necessary adherence level to have optimal HIV protection benefits from oral PrEP and other biomedical products in the pipeline require additional research.

In our primary analysis of PrEP efficacy among women with Nugent scores 7–10 versus Nugent scores of 0–3, assessed in a time-dependent fashion, we saw very similar degrees of protection afforded by PrEP and no statistical difference, although our statistical power to detect an interaction was limited. In other comparisons of markers of vaginal dysbiosis, we had limited power to observe statistical differences in the degree of protection by PrEP. Nonetheless, the HIV incidence rates among women randomized to PrEP were substantially less than those for women on placebo in all subgroups and the hazard ratio estimates for protection from PrEP are statistically significant for nearly all subgroups.

We used microscopy to determine Nugent scores and the presence of BV. This method provides information about the abundance of bacterial morphotypes, but does not identify individual bacterial species (20). In a subset of participants, we had qPCR data available, and we saw a strong correlation between higher Nugent scores and the concentration of *Gardnerella vaginalis*, consistent with previous studies (21). The CAPRISA analysis identified *Gardnerella vaginalis*, detected through metaproteomic methods, as an important species that could disrupt HIV protection from tenofovir 1% gel, prompting our analysis with the *Gardnerella/Bacteroides* component of the Nugent score. However, the score aggregates *Gardnerella* and *Bacteroides* morphotypes, masking the relative predominance of each, which could limit our ability to determine which morphotypes are most present.

Further work to characterize the microbiome and estimate oral PrEP efficacy in the presence of different vaginal microbiome types (e.g. lactobacilli-dominated or anaerobic dysbiosis) are important to confirm or refute our findings and increase our understanding of how the microbiome interacts with topical and systemically delivered PrEP. Specific bacteria are hypothesized to increase HIV risk through inflammatory mechanisms, including *P. bivia, Gemella asaccharolytica, Megasphaera, Mycoplasma hominis*, *Leptotrichia/Sneathia*, and *Eggerthella* species type 1 (13, 28, 29) and the potential role for these bacteria to disrupt oral PrEP efficacy is not known. Another limitation of our work is that we measured Nugent scores annually and some women experience frequent transitions between vaginal microbiota states. More frequent measurement would minimize misclassification and longitudinal pharmacokinetic studies among smaller samples would provide key metabolic data.

The rollout of oral PrEP for HIV prevention to high risk groups is underway in sub-Saharan Africa, including to young women in areas with particularly high HIV burden. BV and abnormal vaginal microbiota are particularly common in these populations. Our results indicate that in the setting of high adherence to PrEP, women with vaginal dysbiosis receive the same high level of protection as women with normal microbiota. Integrating PrEP delivery with other services, such as STI testing and reproductive health care, is the ideal as PrEP delivery programs are developed to scale. Our data are reassuring that there is no need to require that oral PrEP delivery be contingent upon testing for BV or any marker of vaginal dysbiosis, and they also suggest that treatment of BV is unnecessary to gain protective benefits from oral PrEP. As PrEP implementation continues, delivery models that make PrEP available to women with high risk and maximize adherence when there is risk of HIV exposure must be expanded in order to have the greatest impact on reducing HIV incidence.

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Partners PrEP Study Team

University of Washington Coordinating Center and Central Laboratories

Connie Celum (principal investigator, protocol co-chair), Jared M. Baeten (medical director, protocol co-chair), Deborah Donnell (protocol statistician), Robert W. Coombs, Lisa Frenkel, Craig W. Hendrix, Jairam Lingappa, M. Juliana McElrath.

Study sites and site principal investigators

Eldoret, Kenya (Moi University, Indiana University): Kenneth Fife, Edwin Were; Kabwohe, Uganda (Kabwohe Clinical Research Center): Elioda Tumwesigye; Jinja, Uganda (Makerere University, University of Washington): Patrick Ndase, Elly Katabira; Kampala, Uganda (Makerere University): Elly Katabira, Allan Ronald; Kisumu, Kenya (Kenya Medical Research Institute, University of California San Francisco): Elizabeth Bukusi, Craig Cohen; Mbale, Uganda (The AIDS Support Organization, CDC-Uganda): Jonathan Wangisi, James Campbell, Jordan Tappero; Nairobi, Kenya (University of Nairobi, University of Washington): James Kiarie, Carey Farquhar, Grace John-Stewart; Kenya Medical Research Institute, Nairobi, Kenya: Nelly Rwamba Mugo; Tororo, Uganda (CDC-Uganda, The AIDS Support Organization): James Campbell, Jordan Tappero, Jonathan Wangisi.

Data management was provided by DF/Net Research, Inc. (Seattle, USA) and site laboratory oversight was provided by Contract Laboratory Services (University of the Witwatersrand, Johannesburg, South Africa).

Research in context

Evidence before this study

We searched PubMed and abstracts from major international AIDS conferences for efficacy analyses of HIV prevention products published before May 12, 2017, with the terms “HIV prevention,” “PrEP”, and “vaginal dysbiosis” or “bacterial vaginosis,” or “microbiome” with no language restrictions. In conference abstracts, studies showed a difference in the impact of 1% tenofovir gel used as PrEP and suggested that women with vaginal dysbiosis did not receive the same protective benefit of the gel as women with normal microbiota and women with markers of inflammation did not receive protection from PrEP relative to women with no markers of inflammation. Additionally, two studies suggested that *Gardnerella vaginalis* degrades tenofovir. Ours is the first study to report on how bacterial vaginosis may influence the degree to which daily oral PrEP protects women from HIV infection.

Added value of this study

Our findings demonstrate similar rates of HIV protection from oral PrEP among women with BV characterized by microscopy, relative to women with normal microbiota. Other studies have evaluated the effect of vaginal dysbiosis on 1% tenofovir gel and our study contributes data regarding oral PrEP.

Implications of all the available evidence

Our findings are reassuring that the efficacy of daily oral PrEP is unlikely to be modulated by the presence of BV. This is important because oral PrEP needs to be efficacious in the context of BV since BV is often common in settings with high HIV burden. It is also important because oral PrEP is available to women seeking HIV prevention in multiple locations and delivery needs to be accompanied with full information about the efficacy and anything that can reduce efficacy.

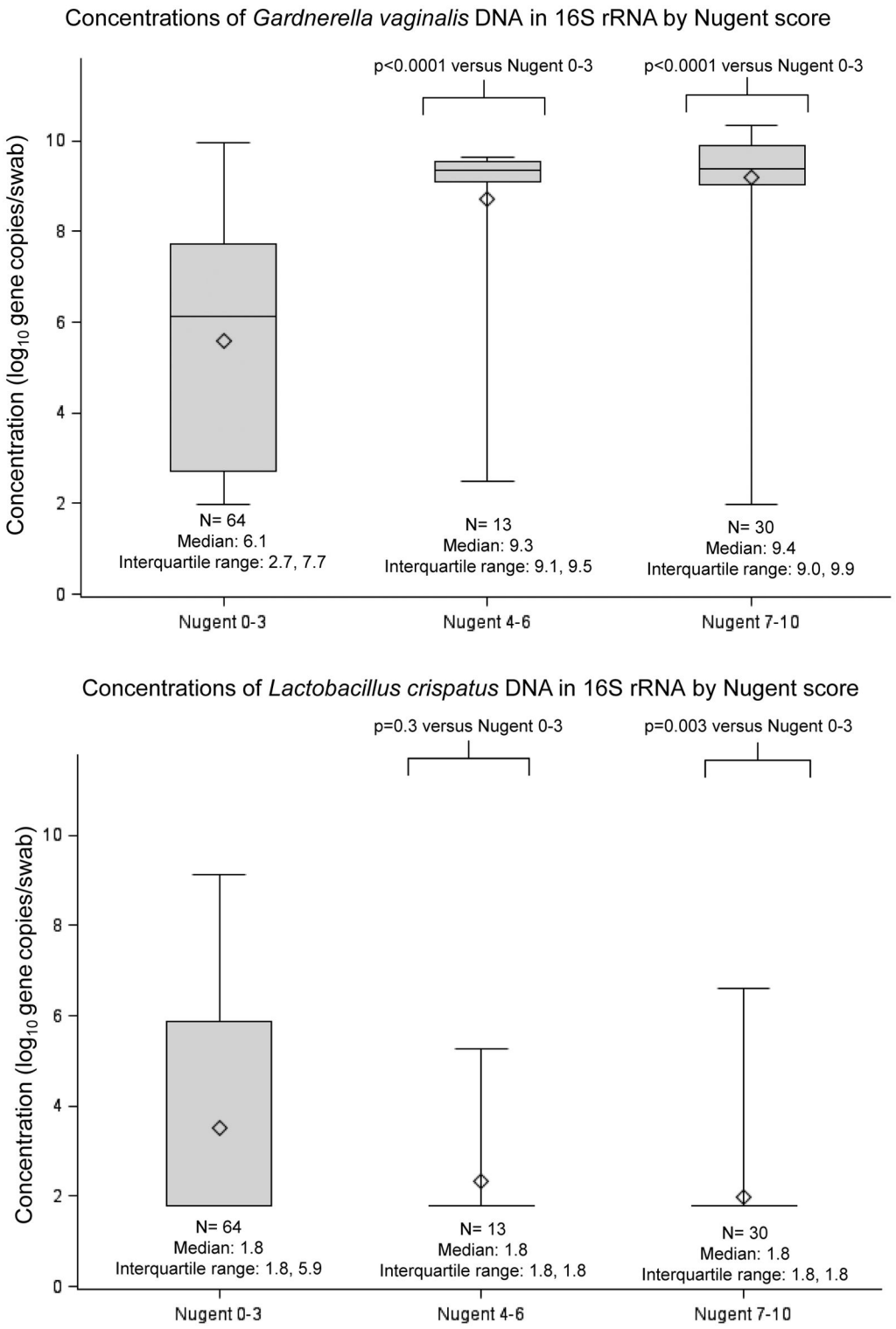

Figure 1. Concentrations of a) *Gardnerella vaginalis* and b) *Lactobacillus crispatus* DNA in 16S rRNA within Nugent score categories

P-values are from Wilcoxon tests comparing the median concentration per category to the reference category (Nugent score 0–3).

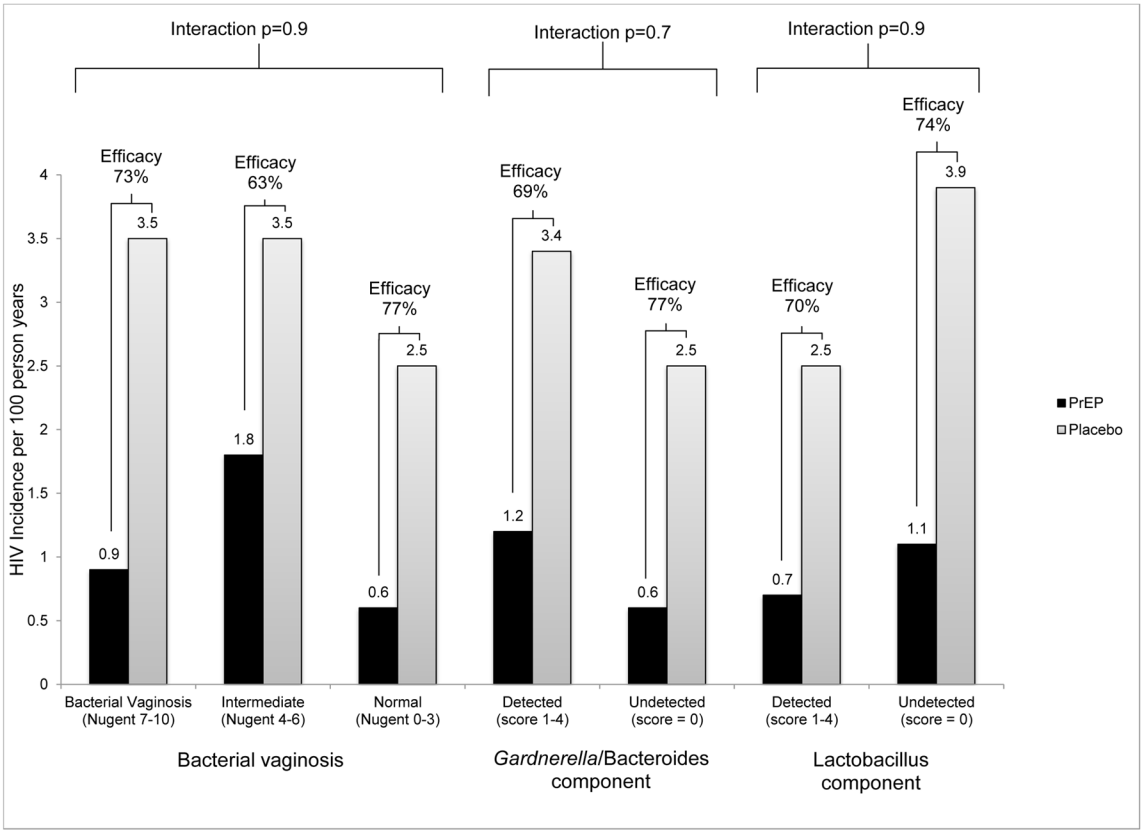

Figure 2. Efficacy of daily oral PrEP for HIV prevention in women with and without vaginal dysbiosis (based on time-varying Nugent scores)

Interaction p-values are from global Wald tests comparing PrEP efficacy across categories of Nugent scores.

Table 1

Characteristics of women

	Baseline Nugent 0–3 n=933	**Baseline Nugent 4–6 n=180**	**Baseline Nugent 7–10 n=357**	**All women n=1470**
	N (%) or Median (IQR)	**N (%) or Median (IQR)**	**N (%) or Median (IQR)**	**N (%) or Median (IQR)**
Demographic characteristics				
Age, yrs	33.0 (28.0–38.0)	33.0 (27.0–39.5)	34.0 (28.0–39.0)	33.0 (28.0–39.0)
Age <25 years	119/933 (12.8)	27/180 (15.0)	48/357 (13.4)	194/1470 (13.2)
Married	922/933 (98.8)	179/180 (99.4)	355/357 (99.4)	1456/1470 (99.0)
Partnership duration, yrs	12.6 (6.3–18.5)	10.9 (5.6–18.8)	11.4 (5.1–18.9)	12.0 (5.9–18.5)
Number of children	3 (1–5)	3 (1–5)	3 (1–4)	3 (1–5)
Behavioral characteristics				
Number of sex acts with study partner, past month	4 (2–7)	5 (3–9)	4 (2–8)	4 (2–8)
Number of unprotected sex acts with study partner, past month	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–0)
Any unprotected sex with study partner, past month	195/933 (20.9)	39/180 (21.7)	106/357 (29.7)	340/1470 (23.1)
Any sex with additional partner, past month	4/933 (0.4)	1/180 (0.6)	3/357 (0.8)	8/1470 (0.5)
Clinical characteristics				
Using hormonal contraception (injectable, oral, or implantable)	383/933 (41.1)	60/180 (33.3)	126/357 (35.3)	569/1470 (38.7)
Infected with gonorrhea, chlamydia, or trichomonas	47/933 (5.0)	29/180 (16.1)	41/357 (11.5)	117/1470 (8.0)
Received medication to treat symptoms common with bacterial vaginosis	82/933 (8.8)	12/180 (6.7)	30/357 (8.4)	124/1470 (8.4)
CD4 count, HIV-1 infected male partner (cells/μL)	452 (352–591)	478 (366–617)	461 (353–576)	459 (354–598)
Viral load, HIV-1 infected male partner (log_{10} copies per mL)	4.1 (3.4–4.7)	4.1 (3.5–4.8)	4.2 (3.4–4.7)	4.1 (3.4–4.7)
Active PrEP arm	604/933 (64.7)	125/180 (69.4)	235/357 (65.8)	964/1470 (65.6)
Nugent score				
Full score (range 0–10)	0 (0–0)	5 (4–6)	8 (8–9)	0 (0–6)
Gardnerella vaginalis/*Bacteroides* component (range 0–4)	0 (0–0)	4 (4–4)	4 (4–4)	0 (0–4)
Lactobacillus component (range 0–4)	4 (4–4)	3 (2–3)	0 (0–0)	4 (2–4)

Table 2

HIV incidence and PrEP efficacy among subgroups of women defined by: a) time-varying Nugent score and score components, and b) baseline Nugent score and score components

	HIV incidence per 100 person years (seroconversions/person years)		PrEP efficacy			
	Placebo arm	**TDF or FTC/TDF arm**	**HR (95% CI)**	**Efficacy (95% CI)**	**p-value for PrEP efficacy**	**Interaction p-value**
Overall	2.8 (28/996.13)	0.9 (17/1936.06)	0.30 (0.16–0.55)	70.49 (45.45–84.03)	<0.001	--
A. Time varying Nugent score and score components						
Full Nugent score						
Nugent score 0–3	2.5 (16/648.74)	0.6 (7/1215.83)	0.23 (0.10–0.57)	76.55 (43.09–90.37)	0.001	0.871
Nugent score 4–6	3.5 (4/114.75)	1.8 (4/225.80)	0.37 (0.08–1.67)	62.72 (−66.59–91.66)	0.196	
Nugent score 7–10	3.5 (7/199.53)	0.9 (4/422.57)	0.28 (0.08–0.94)	72.50 (5.98–91.95)	0.040	
Gardnerella vaginalis/*Bacteroides* score component						
Detected	3.4 (11/321.79)	1.2 (8/653.74)	0.31 (0.12–0.81)	68.62 (19.02–87.84)	0.017	0.652
Undetected	2.5 (16/640.39)	0.6 (7/1209.70)	0.23 (0.10–0.57)	76.72 (43.40–90.42)	0.001	
Lactobacillus score component						
Detected	2.5 (20/784.88)	0.7 (11/1511.10)	0.30 (0.09–1.01)	70.48 (−0.98–91.37)	0.052	0.86
Undetected	3.9 (7/177.30)	1.1 (4/353.10)	0.26 (0.12–0.55)	74.08 (44.62–87.87)	<.001	
B. Baseline Nugent score and score components						
Full Nugent score						
Nugent score 0–3	2.6 (14/543.52)	0.7 (7/1011.49)	0.23 (0.09–0.60)	76.99 (40.11–91.16)	0.003	0.91
Nugent score 4–6	6.3 (5/79.64)	1.0 (2/206.64)	0.16 (0.03–0.81)	84.30 (18.99–96.96)	0.027	
Nugent score 7–10	2.1 (4/191.36)	0.5 (2/386.19)	0.25 (0.05–1.35)	75.19 (−35.49–95.46)	0.108	
Gardnerella vaginalis/Bacteroides score component						
Detected	3.6 (10/276.29)	0.8 (5/601.75)	0.23 (0.08–0.68)	76.87 (32.30–92.10)	0.008	0.881
Undetected	2.4 (13/538.24)	0.6 (6/1001.80)	0.21 (0.07–0.58)	79.36 (42.10–92.64)	0.003	
Lactobacillus score component						
Detected	3.1 (20/652.12)	0.7 (9/1303.10)	0.36 (0.06–2.15)	63.99 (−115.49–93.98)	0.263	0.56
Undetected	1.8 (3/162.41)	0.7 (2/301.21)	0.20 (0.09–0.46)	79.95 (54.47–91.17)	<.001	