Efficacy of oral pre-exposure prophylaxis (PrEP) for HIV among women with abnormal vaginal microbiota: a post-hoc analysis of the randomised, placebo-controlled Partners PrEP Study

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Summary

Background Daily oral tenofovir-based pre-exposure prophylaxis (PrEP) is high efficacious for HIV prevention among women with high adherence. However, the effect of abnormal vaginal microbiota on PrEP efficacy is of concern. We investigated whether bacterial vaginosis modified the efficacy of oral PrEP.

Methods We used prospectively collected data from women in the Partners PrEP Study, a placebo-controlled trial of daily oral PrEP (either tenofovir monotherapy or a combination of tenofovir and emtricitabine) in HIV serodiscordant couples aged 18 years or older in Kenya and Uganda that showed high efficacy in women. We used Cox proportional hazards regression to assess PrEP efficacy among subgroups of women defined by bacterial vaginosis status based on yearly microscopy and Nugent scoring (0–3 indicated healthy microbiota, 4–6 intermediate, and 7–10 bacterial vaginosis). In separate efficacy analyses, we also investigated individual components of the score (ie, detection of *Gardnerella vaginalis* or *Bacteroides* spp and non-detection of *Lactobacillus* spp) as markers of abnormal microbiota.

Findings Of 1470 women (median age 33 years), 357 (24%) had bacterial vaginosis at enrolment. 45 women seroconverted to HIV. The HIV prevention efficacy of PrEP did not differ significantly among women with healthy microbiota (incidence 0·6 per 100 person years in PrEP group and 2·5 per 100 person-years in the placebo group; efficacy 76·55% [95% CI 43·09 to 90·37]), intermediate microbiota (HIV incidence 1·8 per 100 person-years in the PrEP group and 3·5 per 100 person-years in the placebo group; efficacy 62·72% [95% CI –66·59 to 91·66]), or bacterial vaginosis (HIV incidence 0·9 per 100 person-years in the PrEP group and 3·5 per 100 person-years in the placebo group; efficacy 72·50% [95% CI 5·98 to 91·95]; $p_{interaction}$=0·871). PrEP efficacy was not significantly different between women with detected *G vaginalis* or *Bacteroides* spp morphotypes and those without these morphotypes (efficacy 68·62% *vs* 76·72%; $p_{interaction}$=0·652); or between those with *Lactobacillus* spp morphotypes and those without (70·48% *vs* 74·08%; $p_{interaction}$=0·86).

Interpretation Among African women with a high prevalence of bacterial vaginosis and high adherence to PrEP, the efficacy of daily oral PrEP for HIV prevention did not differ significantly among women with abnormal versus healthy vaginal microbiota as defined by Nugent score. These data are reassuring that oral PrEP delivery to women can continue without the need for concurrent testing for bacterial vaginosis or vaginal dysbiosis.

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Introduction

Oral tenofovir-based pre-exposure prophylaxis (PrEP) is a highly effective HIV prevention strategy in women and men. HIV protection is greater than 90% when adherence to the daily dosing regimen is high.[1–3] In 2015, WHO recommended that PrEP be implemented as part of HIV prevention programmes in people with substantial risk of HIV infection, and several countries have now approved tenofovir-based PrEP for HIV prevention. Unlike condoms, which require negotiation between partners for effective use, oral PrEP is discrete, offers the user personal control over HIV prevention, and empowers users and reduces their anxiety.[4] In addition to oral PrEP formulations, topical PrEP preparations, such as a dapivirine-containing vaginal ring and 1% tenofovir gel, were moderately efficacious for prevention in some clinical trials;[5–8] higher efficacy was correlated with evidence of greater adherence.

PrEP did not protect against HIV infection in women in two clinical trials[9,10] in the context of low adherence, and pharmacokinetic data[11] show that vaginal tissue concentrations of tenofovir fall quickly when doses are missed, resulting in suboptimal concentrations of tenofovir and incomplete HIV protection. Biological explanations for these findings have been postulated, including that underlying conditions (eg, inflammation, sexually transmitted infections, bacterial vaginosis, cervical ectopy, exposure to a higher HIV inoculum) could undermine the protective efficacy of PrEP.[12,13] The potential of these biological mechanisms to affect

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Research in context

Evidence before this study

We searched PubMed and abstracts from major international AIDS conferences with the terms "HIV prevention", "PrEP", and "vaginal dysbiosis" or "bacterial vaginosis" or "microbiome" for efficacy analyses of HIV prevention products published in any language on May 12, 2017. Studies presented at conferences suggested that women with vaginal dysbiosis did not receive the same protective benefit from 1% tenofovir gel when used as pre-exposure prophylaxis (PrEP) as women with normal microbiota and that women with markers of inflammation did not receive protection from PrEP compared with women with no markers of inflammation. Additionally, the findings of two studies suggested that *Gardnerella vaginalis* degrades tenofovir.

Added value of this study

We are the first group, to our knowledge, to report how bacterial vaginosis can affect the degree to which daily oral PrEP protects women from HIV infection. Our findings show similar rates of HIV protection from oral PrEP among women with bacterial vaginosis (diagnosed by microscopy) and those with healthy vaginal microbiota.

Implications of all the available evidence

Our findings provide reassurance that the efficacy of daily oral PrEP is unlikely to be affected by the presence of bacterial vaginosis. These findings are important because bacterial vaginosis is common in settings with high HIV burdens. Furthermore, because oral PrEP is available to women seeking HIV prevention in several locations, delivery needs to be accompanied with full information about the efficacy and anything that can reduce efficacy.

PrEP efficacy could vary on the basis of the route of delivery (ie, topical or systemic) among different formulations.

The vaginal microbiota is considered optimal when *Lactobacillus* are the predominant bacterial morphotype and *Gardnerella* spp and *Bacteroides* spp are absent, and suboptimal when non-*Lactobacillus* spp morphotypes, such as *Gardnerella vaginalis*, are predominant on Gram stains. Intermediate vaginal microbiota and bacterial vaginosis have been associated with increased risk of HIV acquisition in several studies.[14] Data suggest that a non-*Lactobacillus* dominant vaginal microbiome could substantially reduce the prevention benefit of 1% tenofovir gel by increasing mucosal inflammation and HIV susceptibility or reducing tenofovir metabolism, or both.[15,16] But so far, no investigations of whether vaginal dysbiosis could affect the HIV prevention efficacy of oral tenofovir-based PrEP have been published.

The Partners PrEP Study was an efficacy trial[17] of daily oral PrEP among east African HIV serodiscordant couples. In the trial, PrEP was efficacious for HIV prevention in both men and women, and in several high-risk subgroups, including women younger than 25 years and women whose HIV-infected partners had a viral load of greater than 50 000 copies per mL.[17] In this post-hoc analysis, we examined whether oral PrEP was less efficacious in women with bacterial vaginosis or microscopic evidence of vaginal dysbiosis on Gram stain than in those with normal vaginal microbiota.

Methods

Study design and participants

The Partners PrEP Study was a phase 3, placebo-controlled, randomised trial of coformulated emtricitabine and tenofovir and single-agent tenofovir for HIV prevention among 4747 HIV serodiscordant couples from nine clinical research sites in Kenya and Uganda. Full procedures and results have been published previously.[1] Eligible HIV-uninfected partners were not infected with hepatitis B virus and had normal renal function, and HIV-uninfected women were encouraged to delay pregnancy until after their study involvement and study drug use. All participants were 18 years or older at enrolment, which began on July 3, 2008. Eligible couples were randomly assigned (1:1:1) to emtricitabine and tenofovir, tenofovir only, or placebo. Standardised questionnaires were administered by interviewers to capture data for demographics at enrolment, and data for sexual behaviour, condom use, and contraceptive use were recorded at every visit. The trial ran until Dec 30, 2012.

In the primary analysis, PrEP efficacy was 67% for tenofovir and 75% for the combination of emtricitabine and tenofovir.[1] Overall, the level of protection afforded by tenofovir did not differ significantly from that afforded by the combination.[18] Among women, the efficacy of tenofovir was 71% (95% CI 37–87) and that of combination PrEP was 66% (28–84).[1]

The study protocol was approved by human participant committees at the University of Washington and all study sites. All participants provided written informed consent in their preferred language. Our analysis includes data collected before July 10, 2011, when the placebo arm of the study was stopped after recommendation from the study's independent data safety and monitoring board because of the substantial protection being provided by both PrEP agents.[1]

Procedures

HIV-uninfected partners attended monthly study visits for HIV testing, prevention counselling, and to receive refills on study drugs. Two HIV rapid tests were done in

parallel at each visit. If at least one rapid test was positive, ELISA testing was done to confirm HIV seroconversion. At enrolment, annual visits, and when clinically indicated, genital examinations were done and genital swab samples were collected from women for bacterial vaginosis testing and screening for *Neisseria gonorrhoeae, Chlamydia trachomatis,* and *Trichomonas vaginalis*.

For bacterial vaginosis, vaginal swabs were rolled onto glass slides at the point of sample collection, air dried, and fixed with absolute methanol. At a laboratory in Mombasa, Kenya, all slides were Gram stained and assessed by microscopy for bacterial vaginosis by two experienced technologists with more than 20 years' experience each. For internal validity, 10% of slides were read by both technologists. For external quality assurance, a panel of slides was sent periodically to the Mombasa laboratory from the University of Washington (Seattle, WA, USA). Bacterial vaginosis was assessed according to Nugent's criteria.[19] The Nugent score is a weighted combination based on microscopic assessment of three bacterial morphotypes: *Lactobacillus* spp (maximum score 4), *G vaginalis* or *Bacteroides* spp combined (maximum score 4), and curved Gram-variable rods (maximum score 2).[19,20,21] Bacterial vaginosis was defined as a Nugent score of 7–10. Women with scores of 0–3 were judged to have healthy microbiota, and those with scores of 4–6 were judged to have intermediate microbiota.

For *N gonorrhoeae, C trachomatis,* and *T vaginalis*, endocervical swabs were collected and tested with the GenProbe Aptima Combo2 (Hologic, San Diego, CA, USA). In addition to diagnostic testing, symptoms of sexually transmitted infections 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 done by staff blinded to PrEP versus placebo assignment. Site laboratory oversight was provided by Contract Laboratory Services (University of the Witwatersrand, Johannesburg, South Africa). In a subset of 107 women from the placebo group, we used results from taxon-specific quantitative PCR analyses assessing concentrations of *G vaginalis* and *Lactobacillus crispatus*,[22] to examine the relationship between the Gram stain results and the bacterial concentration of these key species.

Statistical analysis

The primary exposure of interest was the interaction between PrEP and bacterial vaginosis, and the outcome for all models was incident HIV infection. We combined the single-drug and combination PrEP groups because efficacy was similar in both.[18] In separate analyses, we considered the interaction between PrEP efficacy and women's scores for the *Lactobacillus* and *G vaginalis* and *Bacteroides* spp components of the Nugent score. In these analyses, scores of 0 for an individual component (eg, the *Lactobacillus* component) were deemed

	0–3 (n=933)	4–6 (n=180)	7–10 (n=357)	All participants (n=1470)
Age <25 years	119 (13%)	27 (15%)	48 (13%)	194 (13%)
Married	922 (99%)	179 (99%)	355 (99%)	1456 (99%)
Partnership duration (years)	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	3·0 (1·0–5·0)	3·0 (1·0–5·0)	3·0 (1·0–4·0)	3·0 (1·0–5·0)
Number of sex acts with study partner in past month	4 (2–7)	5 (3–9)	4 (2–8)	4 (2–8)
Number of unprotected sex acts with study partner in past month	0·0 (0·0–0·0)	0·0 (0·0–0·0)	0·0 (0·0–1·0)	0·0 (0·0–0·0)
Any unprotected sex with study partner in past month	195 (21%)	39 (22%)	106 (30%)	340 (23%)
Any sex with additional partner in past month	4 (<1%)	1 (1%)	3 (1%)	8 (1%)
Hormonal contraception used (injectable, oral, or implantable)	383 (41%)	60 (33%)	126 (35%)	569 (39%)
Infected with *Neisseria gonorrhoeae, Chlamydia trachomatis,* or *Trichomonas vaginalis*	47 (5%)	29 (16%)	41 (11%)	117 (8%)
Received medication to treat symptoms common with bacterial vaginosis	82 (9%)	12 (7%)	30 (8%)	124 (8%)
CD4 cell count of HIV-1-infected male partner (cells per μL)	452·0 (352·0–591·0)	478·0 (366·0–617·0)	461·0 (353·0–576·0)	459·0 (354·0–598·0)
Viral load of 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 (65%)	125 (69%)	235 (66%)	964 (66%)
Nugent score				
Full score (range 0–10)	0·0 (0·0–0·0)	5·0 (4·0–6·0)	8·0 (8·0–9·0)	0·0 (0·0–6·0)
Gardnerella vaginalis or *Bacteroides* spp component (range 0–4)	0·0 (0·0–0·0)	4·0 (4·0–4·0)	4·0 (4·0–4·0)	0·0 (0·0–4·0)
Lactobacillus spp component (range 0–4)	4·0 (4·0–4·0)	3·0 (2·0–3·0)	0·0 (0·0–0·0)	4·0 (2·0–4·0)

Data are n (%) or median (IQR). PrEP=pre-exposure prophylaxis.

Table 1: **Demographic, behavioural, and clinical characteristics of participants at baseline, by Nugent score**

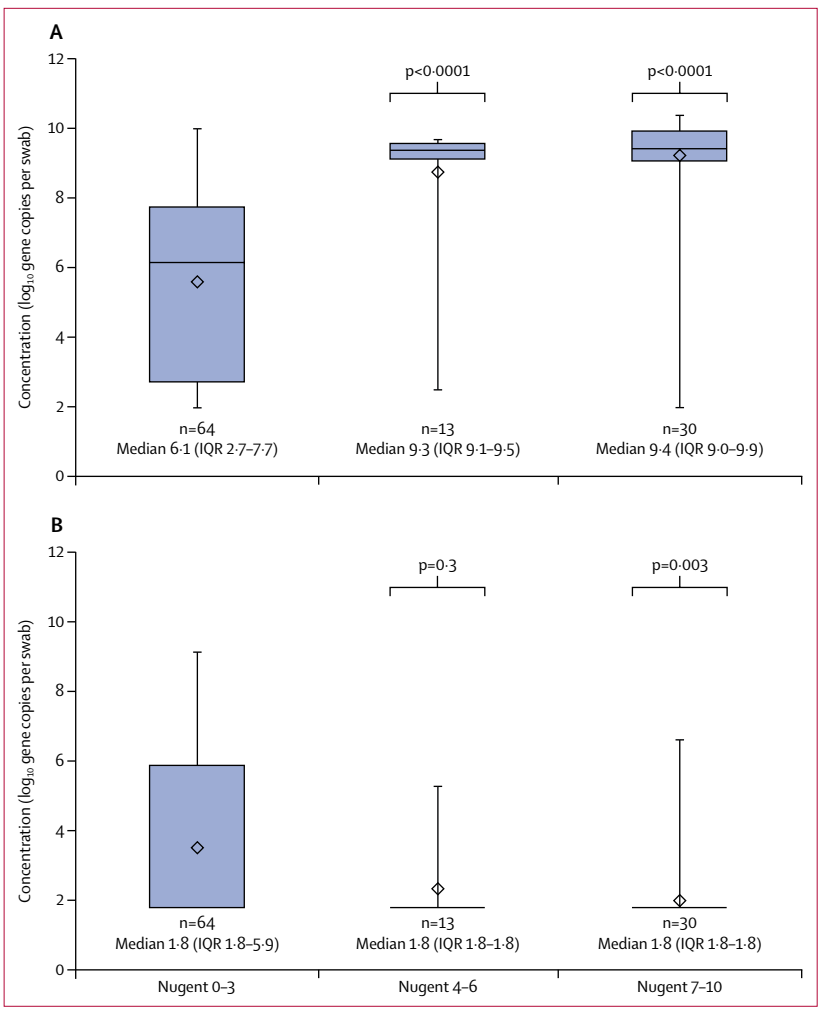

Figure 1: **Concentrations of *Gardnerella vaginalis* (A) and *Lactobacillus crispatus* (B) DNA in 16S ribosomal RNA within Nugent score categories**

p values were calculated with the Wilcoxon tests (Nugent score 0–3 was the reference category). Error bars represent minimum and maximum values.

undetectable and scores of 1–4 were considered detected.

In Cox proportional hazards regression models, we estimated PrEP efficacy (PrEP *vs* placebo, as randomised) among periods categorised by bacterial vaginosis (or *Lactobacillus* spp or *G vaginalis* and *Bacteroides* spp) status. We included an interaction term to assess whether bacterial vaginosis status modified PrEP efficacy, and used the Wald test to calculate interaction p values. Bacterial vaginosis status was a time-dependent variable, with one result carried forward until another result was available. Separately, we used Cox proportional hazards regression models with time-varying covariates to estimate the effect of intermediate microbiota (Nugent score 4–6) or bacterial vaginosis (Nugent 7–10) on HIV incidence; these models were stratified by study group with a priori determined adjustment for age, sexually transmitted infection at enrolment, and time-varying unprotected sex and hormonal contraceptive use. In sensitivity analyses, we substituted baseline (instead of time-varying) bacterial vaginosis, *G vaginalis* and *Bacteroides* spp, and *Lactobacillus* spp status in separate efficacy models. We used the Wilcoxon test to compare the $\log_{10}$-transformed quantitative PCR results across Gram-stain categories. We did all analyses in SAS (version 9.4). Data management was provided by DF/Net Research (Seattle, WA, USA).

Role of the funding source

The funders of the study had no role in study design; data collection, analysis, or interpretation; or writing of the Article. The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

Results

1470 HIV-uninfected women with baseline Nugent scores contributing 2827 person-years were included in our analysis (3·6% of person time was excluded because of missing baseline Nugent score; data not shown). Median age was 33 years (IQR 28–39), nearly all women were married, and they had a median of three children (IQR 1–5; table 1). Women had sex a median of four times (IQR 2–8) with their HIV-infected study partner in the month before enrolment and 1% reported additional partners (table 1).

At enrolment, 357 (24%) of women had Nugent scores of 7–10, 180 (12%) had Nugent scores of 4–6, and 933 (63%) had Nugent scores of 0–3. *Lactobacillus* morphotypes were present in 1175 (80%) of 1469 samples at baseline, and *G vaginalis* or *Bacteroides* spp morphotypes were present in 546 (37%). We obtained Nugent scores on a median of two samples per woman (IQR 2–3). *G vaginalis* or *Bacteroides* spp morphotypes were present in 60 (2%) of 3272 samples from women with Nugent scores of 0–3 at enrolment and follow-up, 540 (91%) of 595 samples from those with Nugent scores of 4–6, and all 1079 samples from those with scores of 7–10. Thus, in general, women tended to sort into groups with greater presence of *Lactobacillus* spp or *G vaginalis* or *Bacteroides* spp, with bacterial vaginosis status highly associated with this grouping (χ^2 p<0·0001 for comparisons of bacterial vaginosis with *Lactobacillus* spp or *G vaginalis* or *Bacteroides* spp). Quantitative PCR showed that, relative to women with Nugent scores of 0–3, women with scores of 4–6 or 7–10 had greater concentrations of *G vaginalis* (p<0·0001 for both comparisons), and women with scores of 7–10 had significantly lower concentrations of *L crispatus* (p=0·003; figure 1). Longitudinally, 573 (48%) of the 1190 women who had at least one Nugent score during follow-up consistently had scores of 0–3, 191 (16%) consistently had scores of 4–10, and 426 (36%) had fluctuating scores during follow-up.

At enrolment, 41 (11%) of 357 women with bacterial

	HIV incidence per 100 person-years (seroconversions/person-years)		PrEP efficacy			$p_{interaction}$*
	Placebo arm	Tenofovir or emtricitabine–tenofovir group	Hazard ratio (95% CI)	Efficacy (95% CI)	p value	
Overall	2·8 (28/996·13)	0·9 (17/1936·06)	0·30 (0·16 to 0·55)	70·49 (45·45 to 84·03)	<0·0001	..
Time-varying Nugent score						
Full Nugent score						
0–3	2·5 (16/648·74)	0·6 (7/1215·83)	0·23 (0·10 to 0·57)	76·55 (43·09 to 90·37)	0·001	0·871
4–6	3·5 (4/114·75)	1·8 (4/225·80)	0·37 (0·08 to 1·67)	62·72 (–66·59 to 91·66)	0·196	..
7–10	3·5 (7/199·53)	0·9 (4/422·57)	0·28 (0·08 to 0·94)	72·50 (5·98 to 91·95)	0·040	..
Gardnerella vaginalis or *Bacteroides* spp component						
Detected	3·4 (11/321·79)	1·2 (8/653·74)	0·31 (0·12 to 0·81)	68·62 (19·02 to 87·84)	0·017	0·652
Undetected	2·5 (16/640·39)	0·6 (7/1209·70)	0·23 (0·10 to 0·57)	76·72 (43·40 to 90·42)	0·001	..
Lactobacillus spp component						
Detected	2·5 (20/784·44)	0·7 (11/1511·10)	0·30 (0·09 to 1·01)	70·48 (–0·98 to 91·37)	0·052	0·86
Undetected	3·9 (7/177·30)	1·1 (4/353·10)	0·26 (0·12 to 0·55)	74·08 (44·62 to 87·87)	0·0005	..
Baseline Nugent score						
Full Nugent score						
0–3	2·6 (14/543·52)	0·7 (7/1011·49)	0·23 (0·09 to 0·60)	76·99 (40·11 to 91·16)	0·003	0·91
4–6	6·3 (5/79·64)	1·0 (2/206·64)	0·16 (0·03 to 0·81)	84·30 (18·99 to 96·96)	0·027	..
7–10	2·1 (4/191·36)	0·5 (2/386·19)	0·25 (0·05 to 1·35)	75·19 (–35·49 to 95·46)	0·108	..
Gardnerella vaginalis or *Bacteroides* spp component						
Detected	3·6 (10/276·29)	0·8 (5/601·75)	0·23 (0·08 to 0·68)	76·87 (32·30 to 92·10)	0·008	0·881
Undetected	2·4 (13/538·24)	0·6 (6/1001·80)	0·21 (0·07 to 0·58)	79·36 (42·10 to 92·64)	0·003	..
Lactobacillus spp component						
Detected	3·1 (20/652·12)	0·7 (9/1303·10)	0·36 (0·06 to 2·15)	63·99 (–115·49 to 93·98)	0·263	0·56
Undetected	1·8 (3/162·41)	0·7 (2/301·21)	0·20 (0·09 to 0·46)	79·95 (54·47 to 91·17)	0·0001	..

PrEP=pre-exposure prophylaxis. *For differences in PrEP efficacy between categories.

***Table 2*: Incidence of HIV and PrEP efficacy among subgroups of women, by Nugent score**

vaginosis, 29 (16%) of 180 with intermediate microbiota, and 47 (5%) of 933 with healthy microbiota were infected with *N gonorrhoeae, C trachomatis,* or *T vaginalis* (table 1). Treatment with metronidazole or other drugs recommended by the US Centers for Disease Control and Prevention and WHO for vaginal symptoms common with bacterial vaginosis was given to 124 women at enrolment, and at 222 (2%) of 9865 quarterly follow-up visits.

45 incident HIV infections were recorded. The overall HIV incidence was 0·9 per 100 person-years in the PrEP group and 2·8 per 100 person-years in the placebo group (table 2). Thus, PrEP had an HIV prevention efficacy 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, the incidence of HIV 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). Women with Nugent scores of 4–6 (adjusted hazard ratio [HR] 1·8 [95% CI 0·8–4·1]; p=0·2) or 7–10 (1·4 [95% CI 0·7–3·0]; p=0·3) did not have a significantly increased risk for HIV acquisition compared with women with Nugent scores of 0–3.

Overall, HIV protection efficacy was similar in women with Nugent scores of 7–10 (72·50%), 4–6 (62·72%), and 0–3 (76·55%; $p_{interaction}$ 0·871; figure 2; table 2). Efficacy estimates were very similar across groups after adjustment for age, sexually transmitted diseases at enrolment, and hormonal contraceptive use (70·56% for scores of 7–10, 64·23% for scores of 4–6, and 83·77% for scores of 0–3; $p_{interaction}$=0·622). Additional adjustment for receiving treatment for symptoms of bacterial vaginosis within the past 3 months did not alter the relation between Nugent category and HIV incidence.

Overall results were similar when looking at the *G vaginalis* or *Bacteroides* spp and *Lactobacillus* spp components of the Nugent score as separate markers of vaginal dysbiosis. PrEP efficacy was 68·62% among women with detectable *G vaginalis* or *Bacteroides* spp and 76·72% among those with undetectable *G vaginalis* or *Bacteroides* spp ($p_{interaction}$ 0·652), and 70·48% among women with detectable *Lactobacillus* spp and 74·08% among those with undetectable *Lactobacillus* spp ($p_{interaction}$ 0·86). When women were classified on the basis of their Nugent score at baseline rather than in a time-dependent fashion during follow-up, PrEP efficacy

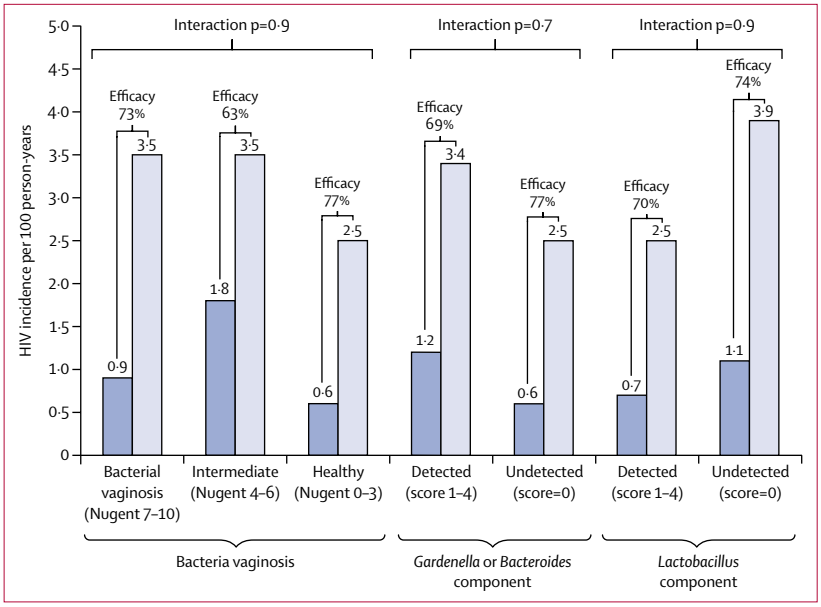

***Figure 2*: Efficacy of daily oral PrEP for HIV prevention in women with and without vaginal dysbiosis based on time-varying Nugent scores**

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

comparisons were similar within categories defined by the full Nugent score and in the *G vaginosis* or *Bacteroides* spp and *Lactobacillus* spp components of the score (table 2).

Discussion

We found no evidence that the protective benefit of daily oral PrEP was reduced in east African women with Gram-stain evidence of bacterial vaginosis or vaginal dysbiosis compared with those with healthy vaginal microbiota. Bacterial vaginosis was common: 24% of participants had bacterial vaginosis by Gram-stain criteria at baseline, and 37% had *G vaginalis* or *Bacteroides* spp morphotypes. Gram-stain results were consistent with quantitative PCR findings in a subset of women, showing that women tended to sort into groups with greater presence of either *Lactobacillus* spp or *Gardnerella* spp. We noted a non-significant association between abnormal vaginal microbiota and increased risk of HIV acquisition, a finding similar to those in other studies.[23] Our data are reassuring that oral PrEP is efficacious for women with abnormal vaginal microbiota.

In CAPRISA 004,[5] a randomised trial of 1% tenofovir gel for HIV prevention among high-risk South African women, primary results showed that the gel had moderate protective benefits (39% efficacy [95% CI 6–60). However, a secondary analysis of CAPRISA 004 data suggests that vaginal dysbiosis, as diagnosed by metaproteomic methods, could modify the protective effect of the gel: women with non-*Lactobacillus*-dominant microbiota received no protective benefits, whereas those with *Lactobacillus*-dominant microbiota did.[16] We used a different method to assess vaginal dysbiosis (Gram staining, supported by quantitative PCR testing in a subset), but the approaches used in the CAPRISA 004 analysis and in our testing would probably have classified women's dysbiosis status similarly. Our results for oral tenofovir-based PrEP do not show the same striking efficacy differences that were reported in CAPRISA 004 with topically applied PrEP.

The metabolic processes for oral PrEP and tenofovir gel are different. The active agents in oral PrEP are systemically distributed to be present in mucosal surfaces and vaginal tissues.[24,25] By 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. Because oral PrEP is absorbed and metabolised systemically, modulation of efficacy by a local mediator, such as bacterial vaginosis or vaginal dysbiosis, is probably less likely.

Adherence to the daily oral PrEP regimen was very high in this cohort, with previous analyses suggesting that more than 80% of participants had plasma concentrations consistent with daily use.[1] Work to understand the pharmacokinetics and pharmacodynamics of daily-use tenofovir-based PrEP has suggested that missing doses might be less of an issue in men who have sex with men than in heterosexual women, because tenofovir is metabolised differently in cervicovaginal and rectal tissue.[9] Further research is needed to understand fully how the genital microbiome could modify this metabolism and the necessary adherence level for optimal HIV protection benefits from oral PrEP and other drugs in development.

In our primary analysis of PrEP efficacy among women with Nugent scores of 7–10 compared with those with Nugent scores of 0–3, assessed in a time-dependent fashion, the degree of protection afforded by PrEP did not differ significantly between groups. However, our statistical power to detect an interaction was limited because our trial was not powered for this subgroup analysis. 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 in women in the PrEP group was substantially less than that in women in the placebo group in all subgroups, and the HR estimates for protection from PrEP were significant for many subgroups.

We used microscopy to determine Nugent scores and the presence of bacterial vaginosis. This method provides information about the abundance of bacterial morphotypes but does not identify individual bacterial species.[20] In a subset of participants we did quantitative PCR, and detected a strong association between high Nugent scores and the concentration of *G vaginalis*, consistent with the findings of previous studies.[21] The CAPRISA analysis identified *G vaginalis* as an important species that could disrupt HIV protection from tenofovir

1% gel, prompting our analysis with the *Gardnerella* spp or *Bacteroides* spp component of the Nugent score. However, the score aggregates *Gardnerella* spp and *Bacteroides* spp morphotypes, masking the relative presence of each, which limits our ability to determine which morphotypes are more common. Further work to characterise the microbiome and estimate oral PrEP efficacy in the presence of different vaginal microbiome types (eg lactobacilli-dominated or anaerobic dysbiosis) are important to support or to refute our findings and to increase understanding of how the microbiome interacts with topical and systemically delivered PrEP. Specific bacteria are postulated to increase HIV risk through inflammatory mechanisms, including *Prevotella bivia, Gemella asaccharolytica, Megasphaera, Mycoplasma hominis*, *Leptotrichia* spp, *Sneathia* spp, and *Eggerthella* spp type 1,[13,28,29] and the potential role that these bacteria have in disruption of oral PrEP efficacy is unknown. Another limitation of our work is that we measured Nugent scores yearly, and some women frequently transition between vaginal microbiota states. More frequent measurement would minimise misclassification, and longitudinal pharmacokinetic studies among smaller samples would provide key metabolic data.

PrEP is being rolled out in sub-Saharan Africa to high-risk groups, including young women in areas with particularly high HIV burden, in whom bacterial vaginosis and abnormal vaginal microbiota are particularly common. Our results suggest that, in the setting of high adherence to PrEP, women with vaginal dysbiosis receive the same high level of protection as women with healthy microbiota. Integration of PrEP delivery with other services, such as testing for sexually transmitted infections and reproductive health care, is the ideal option as PrEP delivery programmes are developed to scale. Our data are reassuring that testing for bacterial vaginosis or any marker of vaginal dysbiosis is unnecessary before oral PrEP delivery. They also suggest that treatment of bacterial vaginosis is unnecessary to ensure protective benefits from oral PrEP. As implementation continues, expansion of delivery through models that make PrEP available to women at high risk and maximise adherence should ensure the greatest effect on reducing HIV incidence.

Contributors

RH and JMB conceived the study. RH did the statistical analyses and wrote the first draft of the Article. RSM and DNF oversaw laboratory technicians who did the analyses of vaginal dysbiosis. All authors contributed critical revisions to the analysis and interpretation, and reviewed the final Article.

Declaration of interests

RSM has received research funding from Hologic, paid as a grant to the University of Washington. All other authors declare no competing interests.

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