Lancet HIV Comment on: ‘Efficacy of oral PrEP for HIV prevention among women with abnormal vaginal microbiota: a randomized, placebo controlled comparison.’

**Title:** Does vaginal dysbiosis modulate HIV PrEP efficacy in women?

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In The Lancet HIV Heffron and colleagues report that the efficacy of daily oral tenofovir disoproxil fumarate (TDF)-based pre-exposure prophylaxis (PrEP) for HIV prevention among African women who participated in the Partners PrEP Study did not differ by vaginal microbiota status (1, 2): the efficacies were 77%, 63%, and 73% for Nugent score categories 0-3 (normal microbiota), 0-6 (intermediate microbiota) and 7-10 (bacterial vaginosis), respectively (Table 1). This analysis was prompted by a report in Science by Klatt and colleagues reporting a large difference of 1% tenofovir (TFV) vaginal gel efficacy against HIV by vaginal microbiota status in the CAPRISA 004 trial (3, 4): the efficacies were 61% in women with >50% *Lactobacillus* (determined by mass spectometry proteomics) compared to only 18% in women with ≤50% *Lactobacillus* (Table 1). However, 1% TFV gel was efficacious against herpes simplex virus type 2 (HSV-2) in both groups.

Prospective cohort studies have previously shown that sexually transmitted infections (STIs), bacterial vaginosis by Nugent scoring, and vaginal candidiasis increase the risk of HIV acquisition in women (5, 6). In recent years, in-depth studies of the vaginal microenvironment suggest that STIs, anaerobic vaginal dysbiosis (defined here as vaginal microbiota not dominated by lactobacilli), vaginal colonisation by bacterial pathobionts (such as streptococci, staphylococci, and *Enterobacteriaceae*) and/or *Candida* species, and exposure to semen all cause immune activation, leading to migration of CD4+ HIV target cells to the cervicovaginal mucosa (7). In addition, vaginal dysbiosis has been associated with weakening of the cervicovaginal barrier via changes in mucus and epithelial layer structure and concentrations of proteolytic enzymes and antimicrobial peptides (8), which might facilitate acquisition of both HIV and HSV-2. Oestrogens promote lactobacilli and strengthen this barrier, whereas use of high dose progestins for contraception may cause vaginal atrophy and associated inflammation due to the induction of hypo-oestrogenism (9). All of these factors are common in women and are intertwined.

TFV-based PrEP studies in women have shown discrepant results, with significant correlations between adherence and HIV efficacy levels in the context of both vaginal and oral administration. The minimum concentrations of tenofovir diphosphate (TFV-DP, the active metabolite) required in the cervicovaginal mucosa, and potentially also draining lymph nodes, to prevent vaginal HIV acquisition are not known, and might differ by levels of vaginal dysbiosis, vaginal immune activation, and HIV exposure. 1% TFV gel application results in higher concentrations in the cervicovaginal mucosa, but lower systemic concentrations, than once daily 300 mg oral TDF (reviewed in 10, 11), but cervicovaginal concentrations are reduced after vaginal sex (12). Dosing aimed at high concentrations might be needed to overcome imperfect adherence and bioavailability, above-average biological HIV vulnerability, and to prevent both HIV and HSV-2 (which has a higher *in vitro* TFV-DP half maximal effective concentration than HIV) (13). The data reported by Heffron et al are reassuring in that they show that oral TDF-based PrEP can achieve high HIV efficacy in women, even in the context of vaginal dysbiosis, when adherence levels are high.

While the pharmacodynamics data and recent data from the VOICE trial (14, 15) favour vaginal administration, the possibility of vaginal dysbiosis-associated bacteria depleting TFV before it is converted by host cells into TFV-DP is more likely with vaginal than oral administration. We think that this hypothesis is biologically plausible and should be further investigated in both epidemiological as well as mechanistic studies. The *in vitro* experiments described by Klatt et al support the hypothesis, but need to be replicated by other laboratories, and should include experiments with bacterial communities in the absence or presence of biofilms. The epidemiological studies presented by Klatt and Heffron were not designed to address the research question and as a result, many of the complexities described above were not adequately addressed. Women with persistent vaginal dysbiosis have increased HIV and HSV-2 vulnerability, and a larger proportion of them may be exposed: vaginal dysbiosis is associated with semen exposure, new sexual partners, and STIs. Furthermore, the factors underlying exposure (such as lack of perception of HIV risk or lack of male partner cooperation with condom use) may also be associated with poor adherence. Importantly, women who have a lactobacilli-dominated microbiota may not be inflammation-free: they may have significant bacterial pathobiont or *Candida* colonisation, both of which were not (Heffron et al) or not sufficiently (Klatt et al) taken into account. Future studies should ideally be longitudinal with frequent relevant assessments of all of these biological factors in all randomised women.

We strongly believe that TDF-based oral PrEP should continue to be rolled-out in communities with a high burden of both HIV and vaginal dysbiosis, and that vaginal PrEP should continue to be developed. Women with vaginal dysbiosis will still be protected if the overall adherence, dosing and timing of TFV applications are right. However, at the same time, vaginal infections and dysbiosis deserve more attention. We have now known for decades that they fuel the HIV epidemic and have other important adverse consequences for women. Many STI control programs, especially those entirely based on syndromic management, could be improved by intensifying screening of at-risk populations and employing point-of-care diagnostics. More efficacious treatments for vaginal dysbiosis should be developed as a matter of urgency. Another potentially very attractive way forward is the development of multipurpose prevention products that deliver antiretroviral drugs in combination with contraceptive hormones into the vagina. This would not only prevent HIV and pregnancy, but would also promote a lactobacilli-dominant vaginal microbiota.

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**Table 1. TDF PrEP HIV and HSV-2 efficacy by study and by vaginal microbiota status**

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| **Study and endpoint** | **Women analysed** | **Inc/100 pyrs**  **control group** | **Inc/100 pyrs TDF group** | **TDF efficacy**  **(95% CI)** |
| Partners PrEP overall – HIV1 | 1,780 | 2.8 | 0.9 | 71% (37-87%) |
| Partners PrEP subanalysis – HIV1 | 1,470 | 2.8 | 0.9 | 70% (45-84%) |
| CAPRISA 004 overall  – HIV2 | 889 | 9.1 | 5.6 | 39% (6-60%) |
| CAPRISA 004 subanalysis – HIV2 | 688 | 7.3 | 4.2 | 57% (36-96%) |
| CAPRISA 004 overall – HSV-22 | 422 | 21.0 | 10.2 | 51% (23-70%) |
| **Study (subanalyses) and endpoint** | **Women analysed** | **TDF efficacy in lactobacilli-dominated (95% CI)** | **TDF efficacy in**  **intermediate**  **(95% CI)** | **TDF efficacy in**  **vaginal dysbiosis**  **(95% CI)** |
| Partners PrEP – HIV3 | 1,470 | 77% (43-90%) | 63% (-67-92%) | 73% (6-92%) |
| CAPRISA 004 – HIV4 | 688 | 61% (11-84%) | N/A | 18% (-77-63%) |
| CAPRISA 004 – HSV-24 | 333 | Cumulative inc:  10.1% | N/A | Cumulative inc:  9.3% |

Abbreviations: CI=confidence interval; HSV-2=herpes simplex virus type 2; inc=incidence; N/A=not applicable; PrEP=pre-exposure prophylaxis; pyrs=personyears of follow-up; TDF= tenofovir disoproxil fumarate.

1. In Partners PrEP, discordant couples in Kenya and Uganda were randomised to daily oral TDF, emtricitabine/TDF or placebo (2). 1,780 women were HIV-negative at baseline, and a baseline Nugent score was available for 1,470 of them (median age 33) (1). The PrEP efficacies of the two drugs were not statistically significantly different and the two drugs were therefore combined in the TDF group.
2. In CAPRISA 004, 889 individual women in South Africa were randomised to 1% TDF vaginal gel or placebo gel (4). Only 688 (mean age 24 and mostly not in stable partnerships) were included in the HIV subanalysis due to unavailability of sufficient quality specimens (3). The HSV-2 subanalysis only included the 422 women who were HSV-2 negative at baseline and sensored HIV seroconverters at the time of their HIV diagnosis (12).
3. In Partners PrEP, vaginal microbiota were assessed by Gram stain Nugent scoring as lactobacilli-dominated (0-3), intermediate (4-6) and vaginal dysbiosis (7-10) (1). The efficacies were 84%, 64%, and 71%, respectively, after adjustment for age, hormonal contraceptive use, and STIs at enrolment.
4. In CAPRISA 004, vaginal microbiota were assessed by mass spectometry as lactobacilli-dominated (>50% relative abundance of lactobacilli) or vaginal dysbiosis (≤50% relative abundance of lactobacilli) (3). The authors state that ‘results were not affected by adjustment for STIs, antibiotics use, DMPA use, condom use, frequency of sex, and number of sexual partners’. We assumed that the HSV-2 subanalysis only included the 333 women who were HSV-2 negative at baseline and had an endline result available, and sensored HIV seroconverters at the time of their HIV diagnosis (3, 13). HSV-2 efficacies could not be calculated because personyears of follow-up in each group were not presented. The p value comparing the cumulative incidence proportions was 0.888.