Blood and Genital Fluid Viral Load Trajectories Among Treated and Untreated Persons With Acute HIV Infection in Malawi

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Background: Persons with acute HIV infection (AHI) are highly infectious and responsible for a disproportionate share of incident infections. Immediate antiretroviral therapy (ART) rapidly reduces blood viral loads (VLs), but genital VLs after ART initiation during AHI are less well described.

Setting: Lilongwe, Malawi, 2012–2014.

Methods: HIV-seronegative and HIV-serodiscordant persons aged ≥18 years were screened for AHI (RNA positive) and randomized to standard of care, behavioral intervention, or behavioral intervention plus short-term ART (raltegravir/emtricitabine/tenofovir) (1:2:2). Persons who were ART eligible under Malawi guidelines could receive first-line therapy. Blood and genital VLs were assessed at weeks 1, 4, 8, and 12. Fisher's Exact test was used to compare viral suppression by ART status.

Results: Overall, 46 persons with AHI were enrolled; of whom, 17 started ART within 12 weeks. Median blood VL at AHI diagnosis was 836,115 copies/mL. At week 12, 7% (1/14) of those who initiated ART had a blood VL of ≥400 copies/mL, compared with 100% (23/23; $P < 0.0001$) of those who did not initiate ART (median VL: 61,605 copies/mL). Median genital VL at week 1 was 772 copies/mL, with 13 of 22 (59%) having VL of ≥400 copies/mL. At week 12, 0 of 10 (0%) of those who initiated ART had genital VL of ≥400 copies/mL, compared with 7 of 15 (47%) of those who did not initiate ART ($P = 0.02$).

Conclusion: Although highly correlated, VLs in blood and genital fluids occupy discrete biological compartments with distinct virologic dynamics. Our results corroborate the dramatic reduction in both compartments after ART initiation. Increasing AHI screening and rapidly initiating treatment is key to interrupting transmission.

Key Words: antiretroviral therapy, acute HIV infection, Malawi

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INTRODUCTION

With highly effective antiretroviral therapy (ART), and the scale-up of universal test and treat strategies, persistent HIV incidence highlights the importance of high-risk transmitters, particularly in established HIV epidemics.[1,2] Despite the temporal brevity of acute HIV infection (AHI), unchecked viral replication and peak viremia[2,3] coupled with frequent missed diagnosis because of the absence of HIV-specific antibodies, AHI plays a disproportionate role in transmissions compared with chronic infection.[1–5] Immediate ART for persons with AHI rapidly reduces viremia,[5–7] and measuring blood viral load (VL) remains the primary clinical biomarker of infectiousness.

Despite most HIV transmissions occurring through sexual contact,[8] little is known regarding changes in genital VLs after ART is initiated during AHI. As in blood, in the absence of ART, genital VLs peak during AHI.[2,9] But plasma and genital fluids occupy distinct biological compartments with different virological and pharmacological dynamics.[4,10,11] Compared with blood, both the viral peaks in AHI and set

points during chronic infection are lower in genital fluids, and the nadir occurs slightly later.[9,12] With the momentum of the *undetectable = untransmissible* (U=U) movement,[13] understanding dynamics of genital VLs after immediate ART initiation among persons with AHI is critical to address transmission and inform patients on the importance of safer sex during peak infectiousness. AHI represents a critical period for behavioral and biomedical intervention to reduce risk of sexual transmission through behavior change and rapid reduction in *genital* VL with ART.

Using a cohort from a 3-arm, randomized, controlled, pilot study evaluating ART and behavioral interventions (BIs) for persons diagnosed with AHI in Lilongwe, Malawi,[5] we examine changes in blood and genital VLs during AHI with and without ART initiation, focusing on the first 12 weeks after AHI diagnosis given this periods' extreme viremia and associated infectiousness.

METHODS

Main study results and methods for AHI screening are described elsewhere.[5,14] Briefly, between June 2012 and January 2014, persons aged ≥18 years who tested HIV seronegative or serodiscordant at 2 HIV testing and counseling clinics and 2 sexually transmitted infections (STIs) clinics were screened for the presence of plasma HIV RNA. HIV serostatus testing was done according to Malawian standard of care with 2 serial rapid tests, Determine HIV-1/2 (Abbott Diagnostics, IL) and Unigold Recombigen HIV-1/2 (Trinity Biotech, Ireland), with a third test for the resolution of discordant results (if positive, persons were considered HIV seropositive). AHI screening was conducted using Abbott RealTime HIV-1 Assay (Abbott Laboratories, Chicago, IL, reportable range of 40–10,000,000 copies/mL) or COBAS AMPLICOR HIV-1 MONITOR test (Roche, Pleasanton, CA, reportable range of 400–750,000 copies/mL). AHI was defined as the presence of HIV RNA in a person with seronegative or serodiscordant antibody results. Participants were randomized to standard of care (SC), sexual BI, or sexual BI and ART (BIA) (1:2:2 ratio) and were followed for 52 weeks.

Viral Load Testing

In addition to blood VLs at screening and enrollment, blood and genital VLs were measured at weeks 1, 4, 8, and 12. Genital VLs were measured at the University of North Carolina using the Abbott RealTime HIV-1 Assay; seminal plasma was diluted 1:1 with normal human plasma before testing, whereas cervical–vaginal lavage had been spun to remove cells before being frozen and did not need pretreatment. Blood VLs were measured as described above. To ensure comparable cutoffs for undetectable VLs, we classify "not detectable" as any VL of <400 copies/mL (2.6 $\log_{10}$ copies/mL). VLs above the limit of quantification were set as the upper limit of quantification, and VLs below the limit of detection were set to half the limit of detection.

ART Initiation

At the time of the study (2012), the relative benefits of immediate ART initiated during AHI had not been established and treatment during AHI was not standard of care. Eligible BIA participants received a 12-week course of raltegravir (400 mg orally, twice daily) and emtricitabine/tenofovir (200/300 mg orally, daily) 3 days after enrollment, with the objective of rapidly reducing VL during peak infectiousness. Persons in the SC and BI arms could receive first-line ART (typically tenofovir, lamivudine, and efavirenz) at any time if medically indicated by Malawian guidelines. Contraindications for study ART included hepatitis B surface antigen positivity, anemia, neutropenia, thrombocytopenia, renal or liver dysfunction, pregnancy, breastfeeding, known hypersensitivity to selected ART, or a need for contraindicated medication. Participants randomized to the BIA arm could receive ART outside of the study even if ineligible for study ART or after completion of the 12-week course if eligible by Malawi ART guidelines. Nonstudy ART receipt was captured by staff inquiry and self-report at each study visit, and all ART use was verified using validated liquid chromatography/tandem mass spectrometry methods.

Statistical Analysis

We describe changes in VL after ART initiation during the 12 weeks following AHI diagnosis and study enrollment. We use Fisher's Exact test and the Mann–Whitney *U* test to assess differences in distributions of categorical and continuous variables, respectively ($\alpha = 0.05$). Longitudinal data are described graphically because of the small sample size. All analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC) or R (version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria).

Ethical Approval

Study procedures were approved by the National Health Sciences Research Committee of Malawi, the Biomedical Institutional Review Board at the University of North Carolina, Chapel Hill, and the National Institute of Allergy and Infectious Diseases' Prevention Science Review Committee. All participants provided informed consent. This study is registered at clinicaltrials.gov (#NCT01450189).

RESULTS

Forty-six participants enrolled (9 [SC], 18 [BI], 19 [BIA]). Twenty-eight (61%) were male, median age was 25 years [interquartile range (IQR): 23–32 years], and median plasma VL at AHI diagnosis was 836,115 copies/mL (IQR: 34,984->10,000,000 copies/mL). High prevalence (11%) of nonnucleoside reverse transcriptase inhibitor resistance mutations was observed, but importantly, no integrase inhibitor resistance was identified.[15]

Seventeen participants received ART within 12 weeks of enrollment (Table 1); of whom, 12 (71%) received study ART and 5 (29%) Malawi first-line therapy. The remaining 29 participants did not receive ART within the first 12 weeks

TABLE 1. Participant Characteristics by ART Status Within12 Weeks of Acute HIV Diagnosis

	No ART	Any ART	Study ART	Malawi First-Line ART
Total	29	17	12	5
Trial arm assignment				
SC arm–No study ART assigned	7 (24)	2 (12)	0 (0)	2 (40)
BI arm–No study ART assigned	16 (55)	2 (12)	0 (0)	2 (40)
BIA arm–Study ART assigned	6 (21)	13 (76)	12 (100)	1 (20)
Sex				
Female	9 (31)	9 (53)	5 (42)	4 (80)
Male	20 (69)	8 (47)	7 (58)	1 (20)
Age, years*	25 (23, 32)	25 (21, 31)	30 (24, 33)	21 (21, 24)
Blood VL at diagnosis (copies/mL)*	1,147,720 (60,820; >10,000,000)	742,370 (13,780; 5,483,080)	746,185 (12,535; 5,276,525)	370,070 (153,125; >10,000,000)
Genital VL at week 1 (copies/mL)*	407 (80; 135,648)	916 (20; 15,148)	628 (20; 15,148)	2,382 (48; 119641)
Weeks to ART initiation*†	—	1 (1, 2)	1 (1, 1)	4 (4, 4)

SC, standard of care; BI, behavioral intervention; BIA: behavioral intervention plus ART.
*Median (IQR).
†Week of visit when ART use was pharmacologically confirmed (eg, ART use already begun).

of enrollment. Seven participants did not receive study ART despite being randomized to the BIA arm because of predefined exclusion criteria.[5] ART was initiated for all participants eligible to receive study ART.

Most participants who initiated ART within 12 weeks of study enrollment (11/17, 65%), initiated within 1 week, all of whom received study drug. Another 5 participants (29%) who initiated ART within 12 weeks, started by week 4 (1 received study drug and 4 received Malawi first-line therapy). The final participant started a Malawi first-line regimen before week 12.

Blood VLs

Early ART effectively reduced blood VLs. At screening, all participants had detectable VLs, but participants who did not receive ART within 12 weeks of enrollment had higher median plasma VLs compared with those who initiated study ART and Malawi first-line regimens (median VL: 1,147,720 copies/mL versus 742,370 copies/mL; $P < 0.0001$; Table 1).

By week 12, only 1 participant (1/14, 7%) who initiated ART had a detectable VL, whereas all participants who did not initiate ART had detectable VLs (23/23, 100%, $P < 0.0001$). Although all VLs were detectable at week 12 among those who did not initiate ART, the median VL fell more than 4-fold from 1,147,720 copies/mL (IQR: 60,820->10,000,000 copies/mL) at screening to 61,605 copies/mL (IQR: 6,361-198,059 copies/mL) at week 12. We observed similar VL patterns in the blood when stratified by sex (Fig. 1A).

Genital VLs

No enrollment genital VL was collected, but a subset of participants in both groups had undetectable genital VL at week 1 (ART: 4/11, 36%; no ART: 5/11, 45%; $P = 1.0$). Among participants who received ART, 12 of 14 had undetectable VL by week 4, 13 of 13 by week 8, and 10 of 10 (100%) had undetectable genital VLs by week 12, whereas among participants who did not receive ART, 8 of 15 (53%) had undetectable genital VLs at week 12 ($P = 0.02$).

When stratified by sex, we observed distinct patterns in genital VL suppression (Fig. 1B). Among those who started ART, 67% (4/6) of women had undetectable genital VLs at week 1, which increased to 100% (6/6) by week 12. Among men who started ART, 0% (0/5) were undetectable at week 1, which increased to 100% (4/4) at week 12.

Among those who did not start ART, 71% (5/7) of women had undetectable genital VLs at week 1 and 80% (4/5) had undetectable VLs at week 12. The median genital VL decreased nearly 2-fold from 270 copies/mL (IQR: 63–407 copies/mL) to 90 copies/mL at week 12 (IQR: 20–165 copies/mL). Among men who did not receive ART, 0% (0/4) had undetectable genital VLs at week 1, which increased to 40% (4/10) by week 12. The median VL decreased more than 8-fold from 242,026 copies/mL (IQR: 82,156–753,945 copies/mL) to 555 copies/mL (IQR: 20–1,636 copies/mL) at week 12.

DISCUSSION

This study of blood and genital VLs among persons with AHI illustrates the dramatic effect of early ART in discrete biological compartments. Our findings support previously documented VL trends of early spikes during AHI but critically expands the period of observation to include the impact of ART on genital VLs when therapy is initiated within weeks of HIV acquisition.

To realize the public health benefits of identifying and treating persons with AHI, early ART must effectively and efficiently reduce blood and genital tract VLs. Rapid reduction of blood VLs after ART initiation during AHI have

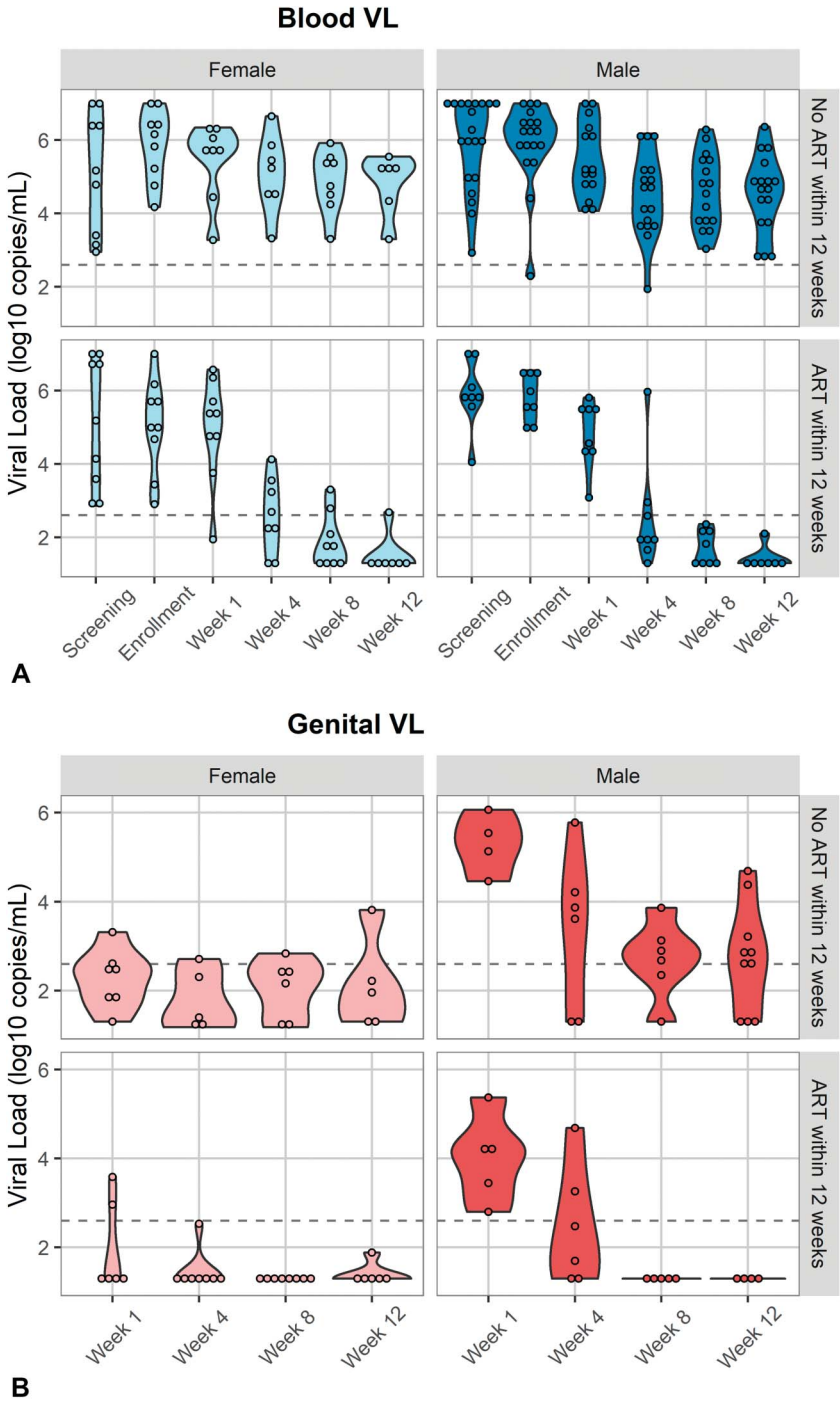

Figure 1. VLs stratified by ART initiation and biological sex, by compartment. Dashed grey line denotes cutoff for undetectable VLs (400 copies/mL or 2.6 log10 copies/mL). Blood VL panel: top left: women who did not initiate ART within 12 weeks; top right: men who did not initiate ART within 12 weeks. Bottom left: women who initiated ART within 12 weeks; bottom right: men who initiated ART within 12 weeks. Genital VL panel: top left: women who did not initiate ART within 12 weeks; top right: men who did not initiate ART within 12 weeks. Bottom left: women who initiated ART within 12 weeks; bottom right: men who initiated ART within 12 weeks.

been reported among men and women in Eswatini (median days to <40 copies/mL: 56),[7] South African women (median days to <20 copies/mL: 16),[6] and Thai men who have sex with men (MSM) (median days to <50 copies/mL: 82).[16] In the United States, the median time to viral suppression (<200 copies/mL) was 131 days after AHI diagnosis, largely reflecting delays in linkage to care.[17] In our study, nearly 95% of participants initiated ART within 4 weeks of study enrollment, 93% achieved blood VL suppression (<400 copies/mL) within 12 weeks (84 days) and 75% achieved <1,000 copies/mL (the accepted threshold by the World Health Organization) within 4 weeks. VLs at diagnosis were lower among those who started ART than those who did not, although not to a degree that would be expected to change time to viral suppression, particularly given the potency of the selected ART regimen.

In the Thai MSM AHI cohort, the median time to <50 copies/mL in the genital tract was 24 days (IQR:

12–79 days). Although our granularity of time to genital VL suppression is limited to 4-week increments, we observed a similar rapid reduction in VL in the genital tract for male participants initiating ART. Additionally, we describe a dramatic reduction in cervical and vaginal viral shedding among women with AHI after 1 month of ART, expanding previously observed effects to an earlier, critical infection stage.[18] Taken together, these findings highlight the potentially monumental public health implications of immediate ART following AHI diagnosis–taking both blood and, perhaps more importantly, genital VL to undetectable levels for the majority of patients within 1 month of treatment.

Our results highlight the importance of combination interventions during AHI, coupling ART with BIs to reduce unprotected sex during the period of persistent infectiousness after starting therapy and before viral suppression. We observed an immediate decrease in sexual risk behaviors, including decreased rates of condomless sex and fewer participants reporting multiple partners, with sustained behavior change across the 1-year follow-up period.[19] Nearly 14% of participants tested positive for chlamydia, gonorrhea, and/or trichomoniasis at their 12-week visit.[5] Co-occurring STIs increase genital VL shedding in both men[20,21] and women.[12] Among HIV-infected Malawian men virally suppressed on ART, nearly 20% of urethritis episodes were associated with genital VL "blipping" despite sustained viremic control.[22] In our cohort, both participants who started ART and had a co-occurring STI at week 12 maintained undetectable genital VLs. Nonetheless, frequent STIs following AHI diagnosis underscores the importance of combined biomedical *and* BIs.

Interestingly, most women had genital VLs of <400 copies/mL at week 1 even without ART, contrasting reports from Zimbabwe/Uganda and Kenya that estimated acute and early genital VLs of 1000 and 2500 copies/swab, respectively.[12,23] Differences may be because of our lavage dilution factor. Regardless, we observed a similar decrease in cervical/vaginal VLs after ART initiation, emphasizing the importance of intervening during the immediate post AHI-diagnosis window.

As high HIV incidence persists, particularly in generalized epidemics across sub-Saharan Africa, identifying persons at greatest risk of transmission and quickly initiating ART is crucial. Persons with AHI, particularly those found in STI clinical settings with documented high-frequency sexual risk behaviors, are responsible for a disproportionate share of new infections compared with persons with chronic infection or advanced HIV.[1,2] Despite sexual transmission being a driving force for HIV infections globally, data are scant regarding genital tract viral dynamics in the presence of immediate, highly-potent ART during AHI. Although limited by a small sample size, our findings will hopefully catalyze a push to implement AHI screening in high-risk settings like STI clinics, and early linkage to ART care as a strategy to interrupt transmission.

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REFERENCES

1. Powers KA, Ghani AC, Miller WC, et al. The role of acute and early HIV infection in the spread of HIV and implications for transmission prevention strategies in Lilongwe, Malawi: a modelling study. *Lancet.* 2011;378:256–268.
2. Miller WC, Rosenberg NE, Rutstein SE, et al. Role of acute and early HIV infection in the sexual transmission of HIV. *Curr Opin HIV AIDS.* 2010;5:277–282.
3. Pilcher CD, Price MA, Hoffman IF, et al. Frequent detection of acute primary HIV infection in men in Malawi. *AIDS.* 2004;18:517–524.
4. Pilcher CD, Shugars DC, Fiscus SA, et al. HIV in body fluids during primary HIV infection: implications for pathogenesis, treatment and public health. *AIDS.* 2001;15:837–845.
5. Miller WC, Rutstein SE, Phiri S, et al. Randomized controlled pilot study of antiretrovirals and a behavioral intervention for persons with acute HIV infection: opportunity for interrupting transmission. *Open Forum Infect Dis.* 2019;6:ofy341.
6. Dong KL, Moodley A, Kwon DS, et al. Detection and treatment of Fiebig stage I HIV-1 infection in young at-risk women in South Africa: a prospective cohort study. *Lancet HIV.* 2018;5:e35–e44.
7. Kerschberger B, Aung A, Mpala Q, et al. Predicting, diagnosing and treating acute and early HIV infection in a public sector facility in Eswatini. *J Acquir Immune Defic Syndr.* 2021;88:506–517.
8. Baeten JM, Kahle E, Lingappa JR, et al. Genital HIV-1 RNA predicts risk of heterosexual HIV-1 transmission. *Sci Transl Med.* 2011;3:77ra29.
9. Pilcher CD, Joaki G, Hoffman IF, et al. Amplified transmission of HIV-1: comparison of HIV-1 concentrations in semen and blood during acute and chronic infection. *AIDS.* 2007;21:1723–1730.
10. Hart CE, Lennox JL, Pratt-Palmore M, et al. Correlation of human immunodeficiency virus type 1 RNA levels in blood and the female genital tract. *J Infect Dis.* 1999;179:871–882.
11. Shepard RN, Schock J, Robertson K, et al. Quantitation of human immunodeficiency virus type 1 RNA in different biological compartments. *J Clin Microbiol.* 2000;38:1414–1418.
12. Morrison CS, Demers K, Kwok C, et al. Plasma and cervical viral loads among Ugandan and Zimbabwean women during acute and early HIV-1 infection. *AIDS.* 2010;24:573–582.
13. Eisinger RW, Dieffenbach CW, Fauci AS. HIV viral load and transmissibility of HIV infection: undetectable equals untransmittable. *JAMA.* 2019;321:451–452.
14. Rutstein SE, Pettifor AE, Phiri S, et al. Incorporating acute HIV screening into routine HIV testing at sexually transmitted infection clinics, and HIV testing and counseling centers in Lilongwe, Malawi. *J Acquir Immune Defic Syndr.* 2016;71:272–280.
15. Rutstein SE, Chen JS, Nelson JAE, et al. High rates of transmitted NNRTI resistance among persons with acute HIV infection in Malawi: implications for first-line dolutegravir scale-up. *AIDS Res Ther.* 2019;16:5.
16. Phanuphak N, Teeratakulpisarn N, van Griensven F, et al. Anogenital HIV RNA in Thai men who have sex with men in Bangkok during acute HIV infection and after randomization to standard vs. intensified antiretroviral regimens. *J Int AIDS Soc.* 2015;18:19470.
17. McNulty M, Schmitt J, Friedman E, et al. Implementing rapid initiation of antiretroviral therapy for acute HIV infection within a routine testing and linkage to care program in Chicago. *J Int Assoc Provid AIDS Care.* 2020;19:2325958220939754.
18. Graham SM, Holte SE, Peshu NM, et al. Initiation of antiretroviral therapy leads to a rapid decline in cervical and vaginal HIV-1 shedding. *AIDS.* 2007;21:501–507.
19. Rucinski KB, Rutstein SE, Powers KA, et al. Sustained sexual behavior change after acute HIV diagnosis in Malawi. *Sex Transm Dis.* 2018;45:741–746.

20. Sadiq ST, Taylor S, Copas AJ, et al. The effects of urethritis on seminal plasma HIV-1 RNA loads in homosexual men not receiving antiretroviral therapy. *Sex Transm Infect.* 2005;81:120–123.
21. Cohen MS, Hoffman IF, Royce RA, et al. Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. *Lancet.* 1997;349:1868–1873.
22. Chen JS, Matoga M, Massa C, et al. Effects of urethritis on HIV in semen: implications for HIV prevention and cure. *Clin Infect Dis.* 2021; 73:e2000–e2004.
23. Lavreys L, Baeten JM, Panteleeff DD, et al. High levels of cervical HIV-1 RNA during early HIV-1 infection. *AIDS.* 2006;20: 2389–2390.