Acute HIV Infections among Men with Genital Ulcer Disease in South Africa

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We investigated acute human immunodeficiency virus (HIV) infection among men enrolled in a genital ulcer treatment trial in South Africa. HIV-negative participants were tested at baseline by HIV RNA polymerase chain reaction and followed up after 1 month to measure HIV seroconversion. There were 228 HIV-negative men at baseline; 10 were positive for HIV RNA, and 8 seroconverted to HIV at day 28. The prevalence of acute HIV among HIV-negative men at baseline was 18 (7.9%) of 228 men (95% confidence interval [CI], 4.4–11.4) and 18 (2.9%) of 615 men (95% CI, 1.6–4.3) in the overall study population. These data highlight the importance of genital ulcer patients in HIV transmission.

Trial Registration. ClinicalTrials.gov identifier: NCT00164424.

Background. Numerous studies have identified higher relative risks of human immunodeficiency virus (HIV) infection with genital ulcer disease than with other sexually transmitted infections (STIs) [1]. In terms of HIV acquisition, the ulcer itself provides a portal of entry, and the inflammation associated with it recruits CD4 T cells as well as other immune cells that serve as targets for HIV infection [2]. The ulcer may facilitate HIV transmission by increasing viral shedding from the genital area [3, 4].

The early (primary) and late stages of human immunodeficiency virus type 1 (HIV-1) have also been associated with high rates of transmission. In 2005, with data from a cohort in Rakai, Uganda, it was estimated that during the first 5 months of infection, the probability of transmission per coital act was 8–10 times higher than it was during asymptomatic infection [5]. Mathematical modeling suggests that HIV-1 primary infection is even more infectious than previously considered (26 times higher than during the asymptomatic period) and may result in clusters of transmission in high-risk populations [6].

Patients with acute HIV are more often identified in STI clinics [7, 8]. We investigated the prevalence of acute HIV among a series of patients with genital ulcer disease in South Africa.

Methods. Data for this analysis arise from an individually randomized, double-blind, placebo-controlled trial of the addition of acyclovir to the syndromic management of genital ulcer disease [9]. This analysis was performed irrespective of randomization status. In brief, men who presented with genital ulcers were recruited at 3 clinics in South Africa. At baseline, a questionnaire was administered to collect information on demographics, sexual behavior, history of STIs, and clinical findings. All patients received free treatment for genital ulcers and other STIs. HIV-Positive participants were referred for appropriate care. Participants were screened for STIs by means of serological and molecular methods [9].

HIV testing followed the South African HIV testing algorithm and was performed on site with 2 sequential rapid HIV tests: Determine (Abbott Laboratories) and Capillus (Trinity Biotech PLC). HIV-negative participants were asked to return on day 28 for a second HIV test. Participants who tested seropositive for HIV at baseline or on day 28 were tested for CD4 cell counts (FACScan flow cytometer, Becton Dickinson Immunocytometry Systems) and HIV-1 plasma viral loads (Amplicor HIV-1 monitor test, version 1.5, Roche Diagnostics System). Western blot testing was performed on all individuals who had seroconverted to HIV by day 28 (New LAV Blot, Bio-Rad). A positive test result was defined as $\geq$2 envelope bands (gp160, gp120, or gp41). An indeterminate result was defined as any reactivity not meeting these definitions.

After data collection was completed, we tested stored baseline sera from antibody-negative participants for the presence of HIV RNA. The COBAS Amplicor Taqman was used for quantitative testing of individual specimens, and had a detection

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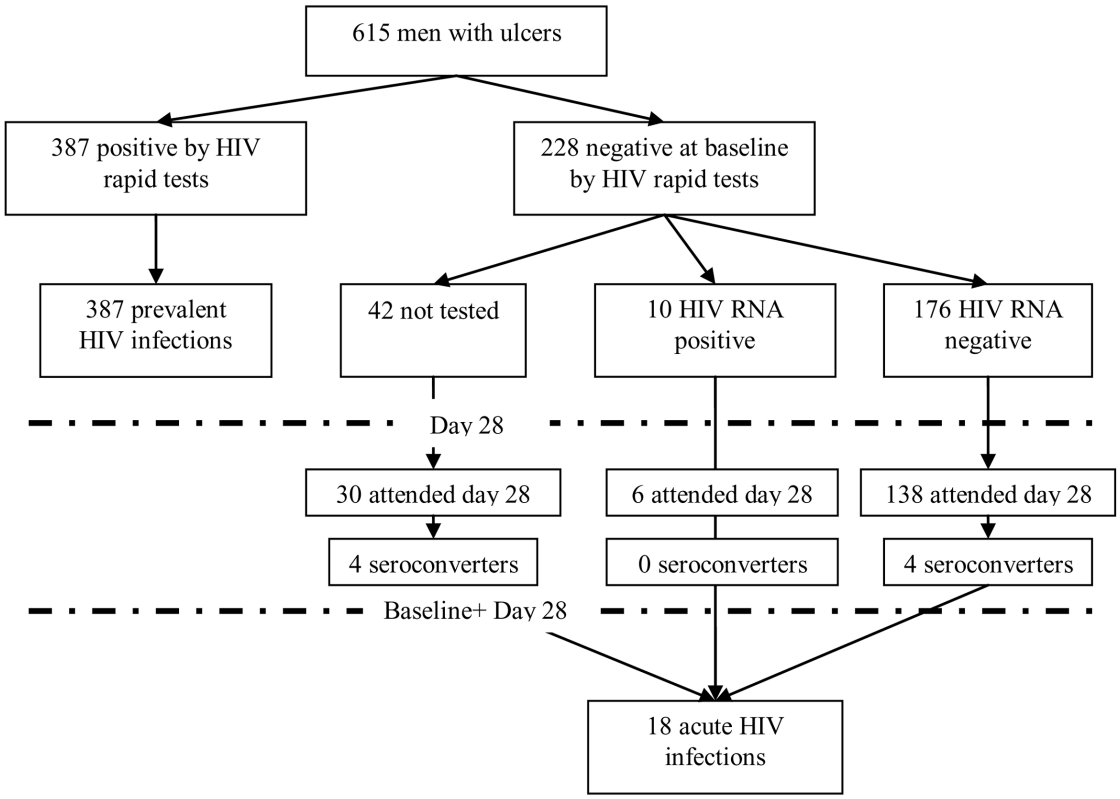

Figure 1. Flow chart of the study population of 615 men, showing the number of prevalent human immunodeficiency virus (HIV) infections, HIV seroconversions, and HIV RNA positive men at baseline and at day 28 visit.

range of 40–1,000,000 copies/mL. There were insufficient sera available to perform HIV RNA testing at the visits on day 28.

An acute HIV infection was defined as either an HIV antibody negative test result and an HIV RNA positive test result at baseline, or a documented HIV seroconversion (HIV-negative antibody test result at baseline and an HIV-positive antibody result by 2 rapid tests at day 28 follow-up).

The prevalence of acute HIV infection among all study participants and among HIV-seronegative participants was estimated together with 95% confidence intervals (CIs). Those who were not tested—because they did not provide consent for their leftover specimens to be stored, had insufficient specimen available, or did not return for the day 28 visit—were considered to be HIV negative. Continuous variables were presented as medians with ranges or means with 95% CIs, and they were compared between groups by using the analysis of variance if distributed normally. To consider the association of selected variables with acute HIV infection, we compared findings in participants with acute HIV with findings in participants susceptible to HIV infection (HIV-negative participants), and we calculated odds ratios (ORs) and 95% CIs. We used multiple logistic regression to construct a multivariate model. Variables that were significant at $P < .10$ were considered for the model and retained if they were significant at $P < .05$.

The study received ethical approval from University of the Witwatersrand, London School of Hygiene and Tropical Medicine, the US Centers for Disease Control and Prevention, the Gauteng Provincial and City of Johannesburg departments of health, and the City of Tshwane Metropolitan Municipality.

Results. A total of 635 men with genital ulcers were eligible to participate in the study, and 615 men consented and were enrolled. The median age of participants was 29 years (range, 25–35 years). Three hundred four (50.4%) participants reported having sex during the last week, and 130 (21.3%) acknowledged having sex while having the genital ulcer. Consistent condom use was reported by 67 (12.4%) participants with regular partners and 44 (22.9%) participants with casual partners.

At baseline, 387 (63.0%) participants tested positive for HIV antibodies. Of 228 HIV-negative participants, 209 consented to have their specimens stored for future testing. There were insufficient or no specimens available for 23 men. Of 186 baseline sera, 10 (5.4%) samples tested positive for the presence of HIV RNA (Figure 1).

Of the 228 HIV-negative participants, 174 (76.3%) returned and were tested for HIV at day 28. Eight (4.6%) tested positive and were classified as HIV-1 seroconverters (Table 1). Western blot results were indeterminate for 7 participants and negative for 1 participant. All 8 had detectable HIV plasma viral loads >30,000 copies/mL at day 28. Three seroconverters reported symptoms that could suggest acute retroviral syndrome, including 1 with high fever, 1 with headache, and 1 with oral lesions. Two of the 8 men had genital lymphadenopathy. Patients who seroconverted had a median CD4 of 340 cells/μL (interquartile range, 260–478) compared with 282 cells/μL (interquartile range, 165–418) among HIV-prevalent cases at baseline. The viral load at the day 28 visit was significantly higher among men who seroconverted (mean, 5.35 log copies/mL; 95% CI, 4.84–5.87) than among men with HIV-prevalent infection (mean, 4.75 log copies/mL; 95% CI, 4.65–4.85; $P = .04$). Of the 10 HIV RNA positive men at baseline, 5 had negative HIV-1 rapid test results at day 28, 1 attended the visit but was not tested, and 4 were not tested because they did not attend the visit on day 28.

The prevalence of acute HIV in the overall study population was 18 (2.9%) of 615 men (based on 10 confirmed positive results for HIV RNA and 8 seroconverters; 95% CI, 1.6–4.3), and among HIV-negative men at baseline the prevalence was 18 (7.9%) of 228 men (95% CI, 4.4–11.4).

We evaluated variables associated with acute HIV RNA among men at risk of infection. Age and urethritis pathogens were not associated with acute HIV. In unadjusted analysis, acute HIV was less common among men with a stable partner (OR, 0.3; 95% CI, 0.1–0.9; $P = .03$) and more common among men with genital lymphadenopathy (OR, 4.1; 95% CI, 1.3–12.9; $P = .01$). Also, acute HIV was more likely among men with ulcers of unknown etiology (OR, 7.7; 95% CI, 2.5–25.0; $P < .001$) and less likely among those with bacterial or mixed etiology (OR, 0.4; 95% CI, 0.1–3.3; $P < .001$) compared with men with herpetic ulcers.

These associations remained after multivariate analysis. Acute HIV was associated with genital lymphadenopathy (OR, 5.9; 95% CI, 1.6–21.8, $P = .01$), ulcers of unknown etiology compared with herpes etiology (OR, 7.7; 95% CI, 2.5–25.0, $P < .001$), and not having a stable partner (OR, 0.3; 95% CI, 0.1–0.9; $P = .04$).

Discussion. This study documents extremely high prevalence of prevalent and acute HIV-1 among men with genital ulcers in South Africa. Eighteen (8%) HIV-negative men had acute HIV-1 infection, which would have gone undetected with conventional HIV antibody testing. In view of the infectiousness of these individuals, genital ulcer patients represent a key target group for HIV prevention.

The prevalence of acute HIV among patients with genital

Table 1. Laboratory Results for Acute Human Immunodeficiency Virus (HIV) Infections among Men with Genital Ulcer Disease, South Africa, 2005–2006

No.	HIV serology baseline	HIV serology day 28	Baseline viral load	Day 28 viral load	CD cell count, cells/μL
HIV seroconverters					
1	Negative	Positive	Negative	98,100	537
2	Negative	Positive	Negative	36,700	296
3	Negative	Positive	NSA	32,300	632
4	Negative	Positive	Negative	182,000	224
5	Negative	Positive	NSA	75,000	214
6	Negative	Positive	NSA	>10,000,000	373
7	Negative	Positive	NSA	>10,000,000	307
8	Negative	Positive	Negative	426,000	419
HIV RNA positive					
1	Negative	Not done	7250	…	…
2	Negative	Negative	>1,000,000	…	…
3	Negative	Not done	1810	…	…
4	Negative	Negative	21,400	…	…
5	Negative	Negative	11,150	…	…
6	Negative	Negative	>1,000,000	…	…
7	Negative	Not done	>1,000,000	…	…
8	Negative	Not done	680,400	…	…
9	Negative	Negative	>1,000,000	…	…
10	Negative	Not done	>1,000,000	…	…

NOTE. NSA, no specimen available; ND, not done.

ulcer disease is higher than what has been found among STI patients in Malawi (4.5%) [7, 8]. Data from Malawi also show that patients with genital ulcerations have almost 6 times the odds of having acute HIV infection compared with other nonulcerative STI patients [7, 10]. Our study showed that acute HIV infections were associated with unknown ulcer etiology, genital lymphadenopathy, and absence of a stable partner.

We were not able to determine the precise timing when patients in our study were infected with HIV-1. At baseline, individuals who were HIV rapid test negative and RNA positive became infected at some point 1–4 weeks prior to the study visit. Those subjects who were negative on the basis of the rapid test results and RNA at baseline but who seroconverted by the 28-day visit became infected ~1 week before to 1 week after the baseline visit. It is likely that the majority of these men were infected with HIV-1 prior to their baseline visit. Although some of these ulcers may have been directly caused by HIV [11], it is more likely that the ulcer itself facilitated the acquisition of HIV.

Men with genital ulcer disease continue to have an important role in HIV transmission in South Africa. High-risk behaviors are common among this population, with many men being sexually active while symptomatic for ulcers and few men reporting consistent use of condoms. The presence of genital ulcers increases susceptibility to HIV among the HIV-negative individuals and infectiousness among the HIV-positive individuals by increased HIV-1 genital shedding [3, 12]. As part of this study, we also documented on a separate analysis that half of the HIV-positive men were shedding HIV-1 from the ulcer [9].

This study had several limitations. The study was not originally designed to look at acute HIV infection; therefore, testing to detect HIV RNA by polymerase chain reaction was performed on stored specimens after data collection was completed; the integrity of the specimens might not have been optimal due to the thawing and freezing cycles. We were not able to conduct HIV RNA testing on all participants at baseline and follow-up due to insufficient sera and imperfect follow-up. To determine the overall number of acute HIV infections, we combined the data from baseline with seroconversions after 1 month follow-up, but as noted above, this includes individuals infected in a window period of 4 weeks prior to 1 week after the baseline visit. We considered all individuals who were not tested to be HIV-negative, which may cause an underestimate in the prevalence of acute HIV and may also affect the analysis of the factors identified as associated with the new infections. Finally, HIV testing was performed with consecutive rapid tests, which have lower sensitivity than do parallel testing, enzyme-linked immunosorbent assay (ELISA), or nucleic acid amplification testing to document acute HIV infection; therefore, we might have failed to document some of the early seroconversions [13].

Acute HIV is most frequently acquired as an STI, and accordingly STI patients are an important group to target with enhanced screening to detect recently acquired HIV infections. Acute HIV infections may be detected by screening for both HIV antibodies and p24 antigen (using a 4th generation ELISA) or by HIV antibodies and HIV RNA polymerase chain reaction [11]. The latter is an expensive approach, and pooling strategies have been employed in some countries. In HIV-prevalent regions of the world, however, these pooling strategies have limited cost-saving potential because of the high burden of HIV disease. There are now available point-of-care tests for antibody and antigen testing, which may be more feasible for resource-poor countries, but these tests need to be validated in clinical settings [14]. Those individuals with negative results should be followed up to monitor seroconversion, and high-risk STI patients should ideally be retested for HIV antibodies between 1 and 3 months.

There is an advantage to detecting very acute HIV infections. Interventions could include early antiretroviral therapy, risk reduction counseling, condom promotion, and enhanced partner testing and counseling [11]. Findings in recent studies that used animal models also suggest that the early stages of HIV infection define a small window of maximum vulnerability for the virus in which there is an opportunity for vaccines or other interventions to prevent or control infection [15]. As part of ongoing HIV prevention efforts, more investment in diagnostic testing needs to be allocated to STI patients to detect and facilitate behavior changes in patients with acute HIV infections as a means of decreasing HIV transmission to uninfected sexual partners.

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