The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Côte d'Ivoire

Peter D. Ghys*†, Katrien Fransen†, Mamadou O. Diallo*, Virginie Ettiègne-Traoré*, Issa-Malick Coulibaly‡, Kouadio M. Yeboué*, Marcia L. Kalish§, Chantal Maurice*, J. Patrick Whitaker*§, Alan E. Greenberg*§ and Marie Laga†

Objective: To measure the frequency and associated factors of cervicovaginal HIV shedding and to determine the impact of sexually transmitted disease (STD) treatment on HIV shedding.

Design: Cross-sectional study with 1-week follow-up.

Setting: Confidential clinic for female sex workers in Abidjan, Côte d'Ivoire.

Participants: A total of 1201 female sex workers.

Interventions: STD treatment based on clinical signs.

Main outcome measures: HIV serostatus; cervicovaginal HIV shedding at enrolment and at 1-week follow-up; STD status at enrolment and at 1-week follow-up.

Results: Cervicovaginal shedding of HIV-1 in HIV-1-seropositive women was more frequent (96 out of 404, 24%) than shedding of HIV-2 in HIV-2-seropositive women [one out of 21, 5%; odds ratio (OR), 6.2; 95% confidence interval (CI), 1.0–261]. Among 609 HIV-1-seropositive or dually seroreactive women, HIV-1 shedding was significantly more frequent in immunosuppressed women [adjusted OR (AOR), 6.3; 95% CI, 3.4–11.9; and AOR, 2.9; 95% CI, 1.6–5.0 for CD4 < 14% and CD4 14–28%, respectively, versus CD4 > 28%], and in women with *Neisseria gonorrhoeae* (AOR, 1.9; 95% CI, 1.2–3.0), those with *Chlamydia trachomatis* (AOR, 2.5; 95% CI, 1.1–5.8), and with a cervical or vaginal ulcer (AOR, 3.9; 95% CI, 2.1–7.4). HIV-1 shedding decreased from 42 to 21% ($P < 0.005$) in women whose STD were cured.

Conclusions: These data help to explain the difference in transmissibility between HIV-1 and HIV-2 and the increased infectiousness of HIV in the presence of immunosuppression and STD. In addition, they lend biological plausibility to arguments for making STD control an integral part of HIV prevention strategies in Africa.

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Keywords: HIV-1, HIV-2, cervicovaginal HIV shedding, Africa, CD4, sexually transmitted diseases, viral load

From the *Projet RETRO-CI, Abidjan, Côte d'Ivoire, the †Institute of Tropical Medicine, Antwerp, Belgium, the ‡Programme National de Lutte contre le SIDA, les MST et la Tuberculose, Abidjan, Côte d'Ivoire and the §Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

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Requests for reprints to: Dr Peter D. Ghys, Projet RETRO-CI, 01 BP 1712, Abidjan 01, Côte d'Ivoire.

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Introduction

A series of epidemiological investigations during the past decade have helped to identify some of the factors that may be responsible for the magnitude of the predominantly heterosexual HIV epidemic in Africa [1,2]. The critical role of commercial sex as a driving force has been extensively studied [3–6], the importance of immunosuppression [7], genital ulcers and other sexually transmitted diseases (STD) [8–12] as risk factors for heterosexual transmission has been demonstrated, and an increased transmissibility of HIV-1 versus HIV-2 has been shown [13]. With polymerase chain reaction (PCR) being applied to detect HIV in various body fluids, researchers have begun to collect biological data to support and explain the observed epidemiological associations.

HIV has been detected in the semen of HIV-1-infected men [14–18], and in some studies seminal HIV shedding has been associated with advanced disease stage and immunosuppression [14,15] and with gonococcal urethritis [15]. A recent study on four men presenting with urethritis suggested that seminal shedding of HIV-1 decreased after treatment of the urethritis [19]. However, few data have been published on the presence of HIV in the cervicovaginal secretions of HIV-seropositive women. Early studies in small numbers of women detected HIV-1 in cervicovaginal secretions using culture on swabbed samples [20,21]. Using culture on cervicovaginal lavage samples, a study of 55 women recruited at various sites in Paris and French Guyana demonstrated HIV-1 in 22% [22]. Using DNA PCR on swabbed samples, a study among 97 Nairobi women attending an STD clinic demonstrated cervical HIV-1 shedding in 33% and found that HIV shedding was more frequent in the presence of oral contraceptive use, pregnancy, cervical ectopy and cervical mucopus [23]. Using the same technique, a study among 92 female sex workers showed cervical HIV-1 in 39% and an association with cervical polymorphonuclear leukocytes [24].

The objectives of the present study were to measure the frequency and associated factors of cervicovaginal HIV shedding among a large number of female sex workers and to determine the impact of STD treatment on HIV shedding.

Methods

A cross-sectional study was conducted from April 1994 through November 1995 at a confidential clinic for female sex workers in Abidjan. The study was approved by the Ethical Committees of the Côte d'Ivoire Ministry of Health (Abidjan, Côte d'Ivoire) and the Institute for Tropical Medicine (Antwerp, Belgium), and the Institutional Review Board of the Centers for Disease Control and Prevention (Atlanta, Georgia, USA). Sex workers were contacted at their places of work through a network of peer educators and an education programme of the Côte d'Ivoire Ministry of Health [25,26]. These women were then invited to come to the clinic for free HIV counseling and testing, STD screening and treatment, and condom distribution.

Clinical evaluation and STD diagnosis

At the initial visit, informed consent was obtained and a questionnaire was administered. A general physical examination including a gynaecological examination was performed. Genital ulcers, vaginal discharge, cervical mucopus and cervical ectopy were diagnosed clinically. All women were screened for *Trichomonas vaginalis*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: a wet mount preparation of vaginal secretions collected from the posterior vaginal fornix was examined for the presence of motile *T. vaginalis*, and endocervical swabs were collected for detection of *C. trachomatis* by enzyme immunoassay (EIA Microtrak, Syva Co, Palo Alto, California, USA) and for culture of *N. gonorrhoeae* on modified Thayer–Martin medium. Samples positive for *C. trachomatis* by EIA were confirmed by a blocking assay. Although EIA are not as sensitive as recently developed amplification tests, the assay used had a comparable sensitivity to other EIA [27]. For consenting women who presented with a genital ulcer that was not felt clinically to have a traumatological or dermatological aetiology (e.g., ulcerated eczema, fungal infection, scabies, folliculitis), material was swabbed from the ulcer base and cultured for *Haemophilus ducreyi* on enriched gonococcal agar, enriched Mueller–Hinton agar and enriched Columbia agar with activated charcoal [28,29]. Material from a second swab was transported in viral transport medium to Antwerp and cultured in Vero cells for herpes simplex virus.

Polymerase chain reaction for HIV in cervicovaginal lavage samples

A cervicovaginal lavage specimen was taken from all consenting women before the taking of cervical swabs for STD assays. It was collected by injecting 10 ml phosphate-buffered saline (PBS) directed at the cervical os and aspirating at least 5 ml lavage fluid after allowing 1 min for pooling. The presence of protein in the lavage was determined semi-quantitatively by dipstick test ('Nephur-test + leuco', Boehringer, Mannheim, Germany). The lavage fluid was examined microscopically in a Neubauer counting chamber (Assistent-Karl-Hecht, Sondheim, Germany) for the presence of spermatozoa, vaginal epithelial cells, white blood cells (WBC) and red blood cells (RBC), and the numbers of cells per 400 μl lavage fluid were determined. After centrifugation at 1000 **g** for 10 min, the lavage

supernatant was separated from the pellet and stored in liquid nitrogen until shipment to Antwerp.

Ultracentrifugation of 4 ml supernatant was performed at 4°C for 2 h at 30 000 rpm. The pellet was then suspended in 20 µl diethyl pyrocarbonate-treated bidistilled water on ice. Detection of cell-free HIV RNA was attempted using reverse transcriptase with in-house random primers. A nested PCR for HIV-1 was performed on lavage samples from HIV-1-seropositive and dually seroreactive (HIV-1 and HIV-2) women, while a nested PCR for HIV-2 was performed on lavage samples from HIV-2-seropositive and dually seroreactive women. The primers used for the HIV-1 PCR have been recently described [30]. Primers used for the HIV-2 PCR amplified a part of the long terminal repeat region: outer sense primer, 5′-GCTGGCA GATTGAGCCCTG-3′ (H2L100); outer antisense primer, 3′-GGGACCAGACAATCCTGGGAA-5′ (H2L200); inner sense primer, 5′-CAGCAC TAGCAGGTAGAGCCTGGG-3′ (H2L101); inner antisense primer, 3′-GGTAGAGAGGATCAGCG GCGG-5′ (H2L201). The primers for HIV-1 and HIV-2 had a sensitivity for African subtypes in peripheral blood mononuclear cells of 96.7 and 91%, respectively [30] (unpublished data). For amplification of the outer fragment, 35 PCR cycles of 30 sec at 94°C, 30 sec at 50°C and 30 sec at 72°C were performed on 50 µl reaction mixture. To amplify the inner fragment, 1 µl of the first PCR round was transferred into 50 µl reaction mixture for a second PCR round and 35 cycles of 30 sec at 94°C, 30 sec at 60°C, and 30 sec at 72°C were performed. Positive reactions were identified by electrophoresis of products on a 2% agarose gel. HIV-1 and HIV-2 shedding was defined in women whose lavage supernatant was positive on the respective PCR assays.

We were concerned that PCR might identify HIV from the male client's ejaculate rather than from the cervicovaginal secretions of the female sex workers themselves. However, spermatozoa were seen in only 6% of lavages of HIV-seropositive women and no association was found between the presence of spermatozoa in the lavage and HIV shedding. Furthermore, PCR for HIV-1 and HIV-2 was also performed on 13 lavage samples from HIV-negative women in which spermatozoa were seen, and only one of these women had HIV-1 detected in the lavage. This woman may either have been recently exposed to HIV-1, or been recently infected without having yet seroconverted. To test for the presence of DNA, a subsample of 140 reverse transcriptase PCR-positive lavages were retested by PCR without using reverse transcriptase, of which 53 (38%) were positive. This finding of viral DNA in the supernatant of the lavage fluid was likely to have resulted from ruptured infected cells. The outcome measure of a positive PCR test on the lavage sample in this study may therefore have been a mixture of both cell-free and cell-associated virions. PCR for both HIV-1 and HIV-2 was also performed on lavage samples from 12 HIV-seronegative women, collected from consecutive HIV-seronegative women attending the clinic during the pilot phase of the study in March–April 1994. All 12 lavages were negative for both HIV-1 and HIV-2. In each ultracentrifugation run a positive control consisting of 10 µl viral supernatant of an HIV-1_{IIIB} coculture in 4 ml PBS was included, all of which were positive.

Serological testing

Serum samples were tested for antibodies to HIV-1 and HIV-2 by a diagnostic algorithm including enzyme-linked immunosorbent assay (Genelavia-mixt, Diagnostics Pasteur, Paris, France), synthetic peptide-based test (PeptiLAV, Diagnostics Pasteur, Paris, France) and Western blot (New-LAV Blot, Diagnostics Pasteur, Paris, France; and HIV Blot 2.2, Diagnostic Biotechnology, Geneva, Switzerland). Anti-treponemal antibodies were detected by *Treponema pallidum* haemagglutination assay (TPHA; Fujirebio, Tokyo, Japan), and a rapid plasma reagin (RPR) test was performed (Macro-Vue, Becton-Dickinson, Cokeysville, Maryland, USA); syphilis was diagnosed in women whose TPHA and RPR tests were both positive.

Lymphocyte subset typing and serum viral load

For all women, a total lymphocyte count was performed using an automated blood analyser (Coulter Counter, Coultronics, Margency, France) and flow cytometry was performed using FACScan (Becton-Dickinson, Erembodegem, Belgium). HIV-1 viral load was determined by nucleic acid sequence-based amplification assay (Organon-Teknika, Durham, North Carolina, USA) on 100 µl serum collected from 132 women who shed HIV-1 and from 132 women who did not shed HIV-1. The threshold viral load that could be detected by this assay in this volume was 1000 copies/ml. Recent data have suggested that the available commercial assays for the quantification of HIV-1 RNA are less sensitive for subtype A, the dominant subtype in Côte d'Ivoire, than for other subtypes [31].

Treatment and follow-up

Free treatment for STD on the day of enrolment in the study was based on clinical signs. Non-herpetic genital ulcers were treated with 2.4 million units benzathine–penicillin intramuscularly and 500 mg erythromycin three times daily for 7 days. Cervical mucopurulent discharge was treated with either 1000 mg azithromycin, or with 500 mg ciprofloxacin and 7 days of twice daily 100 mg doxycycline, or with 2 g intramuscular spectinomycin and 7 days of four times daily 500 mg erythromycin if women were pregnant or breastfeeding.

Results of laboratory tests, HIV/AIDS post-test counselling and additional treatment (if indicated) were

given during a second visit 7 days (range, 5–15 days) after enrolment. During this visit, a second gynaecological examination was performed for HIV-seropositive women, another cervicovaginal lavage specimen was taken, and sampling for *N. gonorrhoeae*, *C. trachomatis* and *T. vaginalis* was repeated.

Statistical analysis

Data were analysed using the Epi-Info (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) and SAS (Cary, North Carolina, USA) statistical packages. For univariate analysis of cross-sectional data, odds ratio (OR) was used as the measure of association and its 95% confidence interval (CI) was calculated by parametric or exact methods. For ordered categorical variables, the χ^2 test for linear trend was used. For the comparison of the proportion of women with HIV-1 and HIV-2 shedding among those with dual seroreactivity, McNemar's χ^2 test for paired data was used. For the analysis of risk factors for shedding of HIV, HIV-1-seropositive and dually seroreactive women were grouped because the rate of HIV-1 shedding among dually seroreactive women was very similar to that among HIV-1-seropositive women (see Results). To assess the independent associations of various factors with HIV shedding, logistic regression was performed using models that included all variables that were significantly associated in the univariate analysis; significance testing ($P < 0.05$) was based on Wald's test. Differences in serum viral load between HIV-1 shedders and non-shedders were assessed by the Wilcoxon rank-sum test; serum viral load results below threshold (1000 copies/ml) were considered as 500 copies/ml (halfway between zero and the threshold of 1000) to facilitate calculations. For the comparison of the proportion of women with HIV-1 shedding at the enrolment visit and the proportion of women with HIV-1 shedding at the follow-up visit, McNemar's χ^2 test for paired data was used.

Results

Overall, 1201 women were enrolled between April 1994 and November 1995. These women had a median age of 27 years (range, 14–68 years), had been working for a median of 24 months (range, 1–408 months) and received a median of 1000 francs (Franc de la Coopération Financière d'Afrique; FCFA; US$ 1 = 500 FCFA) from their last client (range, 75–50 000 FCFA). The overall HIV seroprevalence among the 1079 women who consented to HIV testing was 61%: 428 (39.7%) were HIV-1-seropositive, 21 (1.9%) were HIV-2-seropositive, and 209 (19.4%) were dually seroreactive to HIV-1 and HIV-2. Among 658 HIV-seropositive women, lavages were obtained from 630 (96%) women at the enrolment visit, and from 443 of these women at the 1-week follow-up visit (70% follow-up).

Table 1. Cervicovaginal shedding of HIV-1 and HIV-2 by HIV serostatus in female sex workers.

	Serostatus [n (%)]		
	HIV-1	HIV-2	HIV-1/HIV-2*
	(n = 404)	(n = 21)	(n = 205)
HIV-1 shedding	96 (24)	ND	53 (26)‡
HIV-2 shedding	ND	1 (5)	6 (3)‡

*Dually seroreactive to HIV-1 and HIV-2. $P = 0.06$, cervicovaginal shedding of HIV-1 in HIV-1-seropositive women compared with shedding of HIV-2 in HIV-2-seropositive women. ‡$P < 0.001$, cervicovaginal shedding of HIV-1 compared with shedding of HIV-2 in dually seroreactive women. ND, Not determined.

Prevalence of shedding by serostatus

Cervicovaginal shedding of HIV-1 in HIV-1-seropositive women was more frequent (96 out of 404, 24%) than shedding of HIV-2 in HIV-2-seropositive women [one (5%) out of 21; OR, 6.2; 95% CI, 1.0–261; Table 1]. Among dually seroreactive women, shedding of HIV-1 (53 out of 205, 26%) was significantly more frequent than shedding of HIV-2 [six (3%) out of 205; $P < 0.001$], and HIV-2 shedding was more frequent, although not significantly, in women who also shed HIV-1 [five (9%) out of 53] than in women who did not [one (1%) out of 152; OR, 9.1; 95% CI, 0.7–479].

Factors associated with HIV-1 shedding

Among 609 HIV-1-seropositive or dually seroreactive women, HIV-1 cervicovaginal shedding at enrolment was associated in the univariate analysis with immunosuppression, *N. gonorrhoeae*, *C. trachomatis*, cervical or vaginal ulcer, cervical mucopus and ulcerated genital eczema (Table 2). HIV-1 shedding was not significantly associated with syphilis, *T. vaginalis*, vulvar ulcer, vaginal discharge, pregnancy, cervical ectopy, age, contraceptive pill use or condom use. HIV-1 shedding was also significantly associated with the following lavage characteristics: a positive dipstick test for protein [56 (48%) out of 117 versus 93 (19%) out of 490; OR, 3.9; 95% CI, 2.6–5.9], RBC count [45 (46%) out of 98, 33 (28%) out of 118, and 71 (18%) out of 391 for ≥ 10, 1–9 and 0 RBC, respectively; $P < 0.05$] and WBC count [57 (42%) out of 136, 76 (24%) out of 321, and 16 (11%) out of 150, for ≥ 100, 10–99 and < 10 WBC, respectively; $P < 0.05$], but not with the presence of spermatozoa or vaginal epithelial cells. Lavage characteristics and cervical mucopus were not included in the logistic regression model because they are biological factors on the causal pathway between STD, the epidemiological risk factor under study, and cervicovaginal HIV shedding. In this model, HIV-1 shedding remained significantly associated with immunosuppression, *N. gonorrhoeae*, *C. trachomatis*, and cervical or vaginal ulcer (Table 2). Among women

Table 2. Assessment of the association between potential risk factors and cervicovaginal HIV-1 shedding in HIV-1-seropositive and dually seroreactive women.

Potential risk factors	No./total (%) with HIV-1 shedding	OR (95% CI)	AOR (95% CI)*
Immunosuppression			
CD4 percentage			
>28%	20/168 (11.9)	1.0	1.0
14–28%	76/304 (25.0)	2.5 (1.4–4.4)	2.9 (1.6–5.0)
<14%	47/122 (38.5)	4.6 (2.5–8.8)	6.3 (3.4–11.9)
Sexually transmitted agents			
Neisseria gonorrhoeae			
Negative	80/403 (19.9)	1.0	1.0
Positive	67/194 (34.5)	2.1 (1.5–3.1)	1.9 (1.2–3.0)
Chlamydia trachomatis			
Negative	132/563 (23.5)	1.0	1.0
Positive	13/27 (48.2)	3.0 (1.4–6.4)	2.5 (1.1–5.8)
Trichomonas vaginalis			
Negative	103/415 (24.8)	1.0	NI
Positive	46/194 (23.7)	0.9 (0.6–1.4)	
Syphilis			
Negative	110/471 (23.4)	1.0	NI
Positive	39/138 (28.3)	1.3 (0.8–2.0)	
STD-related syndromes and signs			
Cervical or vaginal ulcer			
Negative	120/557 (21.5)	1.0	1.0
Positive	29/52 (55.8)	4.6 (2.7–7.9)	3.9 (2.1–7.4)
Vulvar ulcer			
Negative	134/558 (24.0)	1.0	NI
Positive	15/51 (29.4)	1.3 (0.7–2.5)	
Cervical mucopus			
Negative	103/487 (21.2)	1.0	NI
Positive	46/122 (37.7)	2.3 (1.5–3.4)	
Vaginal discharge			
Negative	59/241 (24.5)	1.0	NI
Positive	90/368 (24.5)	1.0 (0.7–1.5)	
Other conditions and clinical signs			
Ulcerated genital eczema			
Negative	139/591 (23.5)	1.0	NS
Positive	10/18 (55.6)	4.1 (1.7–9.8)	
Pregnant			
No	144/589 (24.5)	1.0	NI
Yes	5/19 (26.3)	1.1 (0.4–3.1)	
Cervical ectopy			
Negative	144/580 (24.8)	1.0	NI
Positive	5/29 (17.2)	0.6 (0.2–1.7)	
Demographic and behavioral			
Age (years)			
10–19	14/61 (23.0)	1.0	NI
20–29	58/278 (20.9)	0.9 (0.4–1.8)	
30–39	63/209 (30.1)	1.5 (0.7–3.0)	
40–49	13/53 (24.5)	1.1 (0.4–2.8)	
50–59	0/4 (0.0)	0.0 (0.0–5.7)	
Contraceptive pill use			
No	138/571 (24.2)	1.0	NI
Yes	9/32 (28.1)	1.2 (0.6–2.7)	
100% condom use on last working day			
No	102/399 (25.6)	1.0	NI
Yes	47/210 (22.4)	0.8 (0.6–1.2)	

*Derived from logistic regression model with all factors significantly associated in univariate analysis except for lavage characteristics and cervical mucopus (see text for rationale). STD, Sexually transmitted diseases; OR, odds ratio; CI, confidence interval; AOR, adjusted OR; NS, not significant ($P > 0.05$); NI, not included in the model.

with a cervical or vaginal ulcer, the frequency of shedding was higher, although not significantly, in women with chancroid than in those without [14 (67%) out of 21 versus 12 (44%) out of 27; OR, 2.5; 95% CI, 0.8–8.2] and was similar in women with and without herpes [four (57%) out of seven versus 19 (53%) out of 36; OR, 1.2; 95% CI, 0.2–6.2].

Persistence of HIV shedding and effect of STD treatment

Among 108 women who shed HIV-1 at enrolment, 43 (40%) were still shedding HIV-1 at the follow-up visit; among 324 women who did not shed HIV-1 at enrolment, only 47 (15%) were shedding HIV-1 at the follow-up visit (OR, 3.9; 95% CI, 2.3–6.6). Among

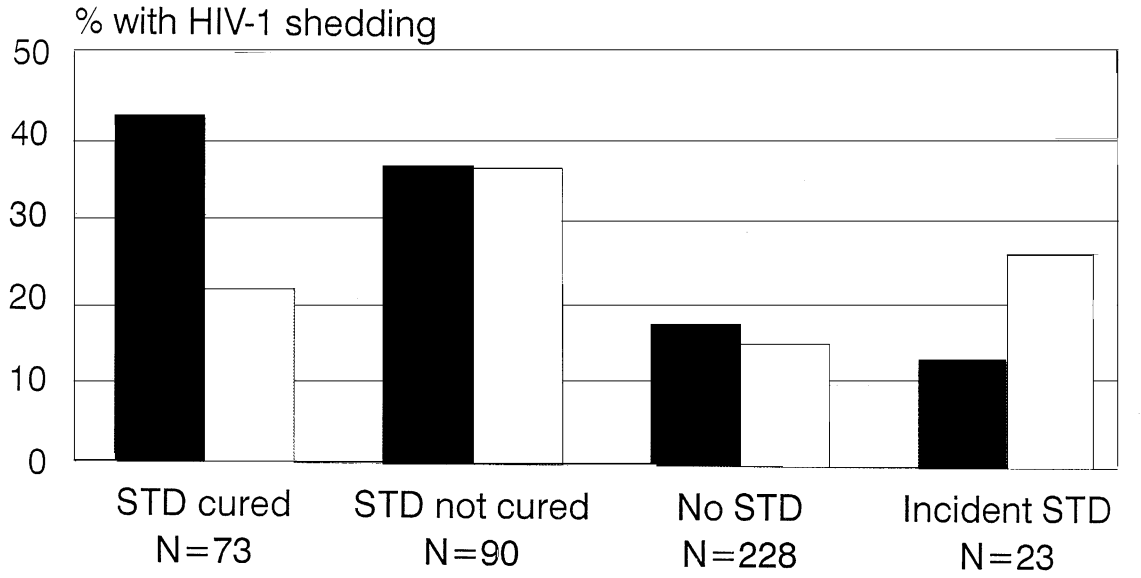

Fig. 1. Cervicovaginal HIV-1 shedding by sexually transmitted disease (STD) status in HIV-1-seropositive and dually seroreactive women. Twin bars represent the proportion of HIV-1-seropositive and dually seroreactive women with cervicovaginal HIV-1 shedding, stratified by STD status at enrolment (dark bars) and at the 1-week follow-up visit (light bars). Definitions of STD status are given in the text. The difference in the proportion of women with HIV-1 shedding between the enrolment visit and the follow-up visit was statistically significant for the STD cured group (42 versus 21%; $P < 0.005$), although it was not for the other three groups.

four women who shed HIV-2 at enrolment, two (50.0%) were shedding HIV-2 at the follow-up visit; among 166 women who did not shed HIV-2 at enrolment, two (1.2%) were shedding HIV-2 at the follow-up visit (OR, 82; 95% CI, 3.5–1510).

Of 432 HIV-1-seropositive and dually reactive women with lavage at both enrolment and follow-up visits, 414 (96%) women had a complete assessment for the presence of cervical or vaginal ulcer and cervical infection with *N. gonorrhoeae* and *C. trachomatis*. In Figure 1, the proportion of these women who shed HIV-1 is shown stratified by the presence of an STD. Among the 73 women whose STD at enrolment was cured at the follow-up visit, the proportion with HIV-1 shedding decreased from 42 to 21% ($P < 0.005$). Among the 90 women who had an STD at enrolment that was not cured at the follow-up visit, the proportion with shedding was 36% at both visits. Among the 228 women who had no STD at either visit, the proportion with shedding was 17% at enrolment and 15% at the follow-up visit ($P > 0.5$). Among the 23 women who had no STD at enrolment, but had an incident STD at the follow-up visit, the proportion with shedding was 13% at enrolment and 26% at the follow-up visit ($P = 0.5$). Among women who were cured of their STD, the proportion of women with HIV-1 shedding decreased significantly for *N. gonorrhoeae* [from 35 (42%) out of 83 to 20 (24%) out of 83; $P < 0.01$], and also decreased, although not significantly, for *C. trachomatis* [from eight (50%) out of 16 to six (38%) out of 16] and for cervical or vaginal ulcer [from three (60%) out of five to one (20%) out of five].

Association of HIV-1 shedding and immunosuppression with serum HIV-1 viral load

HIV-1 serum viral load was significantly higher among the 132 women who shed HIV-1 (median, 3050 copies/ml; range, < 1000–310 000 copies/ml) than among the 132 women who did not shed HIV-1 (median, 1100 copies/ml; range, < 1000–220 000 copies/ml; $P = 0.02$; Fig. 1). In women who did and did not shed HIV-1, serum viral load was associated with immunosuppression: among women who shed HIV-1, the proportion with ≥ 1000 copies/ml was 72, 62 and 30% for women with CD4 percentages of < 14%, 14–28%, and > 28%, respectively; among women who did not shed HIV-1 the proportion with ≥ 1000 copies/ml was 93, 56 and 24% for women with CD4 percentages of < 14%, 14–28%, and > 28%, respectively (both $P < 0.05$). HIV-1 serum viral load was no longer significantly associated with cervicovaginal HIV-1 shedding when stratifying by level of immunosuppression (Fig. 2). Inclusion of HIV-1 serum viral load in the above-mentioned logistic regression model confirmed the absence of a statistically significant association between HIV-1 serum viral load and cervicovaginal HIV-1 shedding.

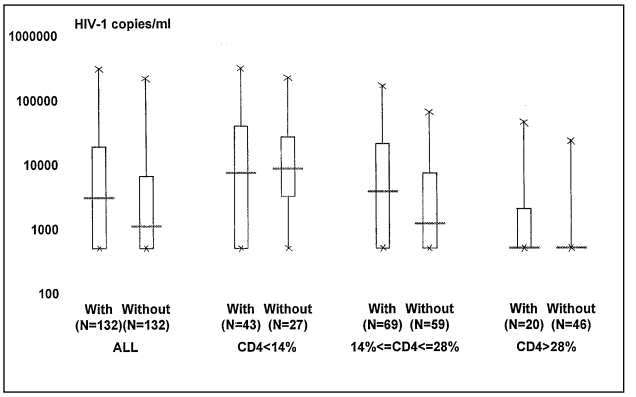

Fig. 2. HIV-1 serum viral load in women with and without cervicovaginal HIV-1 shedding. The distribution of HIV-1 serum viral load among HIV-1-seropositive and dually seroreactive women is shown in paired boxplots, representing women with and without cervicovaginal HIV-1 shedding. The dark line in the middle of the box represents the median, the extremes of the box represent 25th and 75th percentiles, and crosses represent the extreme values. The overall difference in the distribution of HIV-1 serum viral load between women with and without cervicovaginal HIV-1 shedding was statistically significant ($P = 0.02$); however, the difference was not statistically significant within the CD4 percentage strata ($P = 0.4$, 0.2 and 0.4, respectively).

Discussion

We have demonstrated in a large population of HIV-infected female sex workers in Abidjan that cervicovaginal shedding of HIV-1 is more frequent than HIV-2, is associated with immunosuppression and with ulcerative and non-ulcerative STD, and decreases significantly following treatment of STD. This study therefore provides important biological support for epidemiological observations that have been made regarding the heterosexual transmission of HIV in Africa.

Previous studies have demonstrated HIV-1 infection in 91–100% and HIV-2 infection in 33–71% of dually reactive individuals [32–34]. Even if we assume that among the dually reactive women in this study only 91% were infected with HIV-1 and only 33% were infected with HIV-2, shedding of HIV-1 would remain statistically significantly more frequent than shedding of HIV-2. The higher rate of HIV-1 shedding than of HIV-2 shedding may help to explain the higher heterosexual transmission rate of HIV-1 compared with HIV-2 [13]. Although it has been hypothesized that the higher transmissibility of HIV-1 than HIV-2 is due to lower HIV-2 viral load [35,36], this was not confirmed in a community-based study of HIV-2 viral load in Guinea-Bissau [37]. The present study indicates that the increased frequency of cervicovaginal HIV-1 compared with HIV-2 shedding may ultimately be responsible for the increased transmissibility of HIV-1. Differences in HIV-1 shedding by subtype [38] could not be addressed in the present study, since all 16 strains subtyped were subtype A (unpublished data) and recent subtyping of 109 strains from other populations in Abidjan has also found all strains to be subtype A [39].

While cervicovaginal shedding of HIV was intermittent, it was not random, since women who shed HIV at enrolment were significantly more likely to shed HIV at the follow-up visit, and since additional risk factor analyses performed for HIV-1 shedding at the follow-up visit also showed that HIV-1 shedding was associated with immunosuppression, cervical or vaginal ulcer, gonorrhoea and chlamydial infection.

The results of this study provide a plausible explanation for why immunosuppression has been shown in several epidemiological studies to be a risk factor for heterosexual HIV transmission [7,40]. One possible explanation for an increased HIV transmission with immunosuppression is because of an increased circulating viral load. However, in the present study, no independent association was found between cervicovaginal HIV-1 shedding and serum viral load, when controlling for the level of immunosuppression. These data suggest that local factors such as STD, as discussed below, may be more important than circulating viral load in determining transmissibility, and are consistent with those from previous studies [41,42].

Perhaps the most important finding of this study was the strong relationship between cervicovaginal shedding and several STD, namely gonorrhoea, chlamydial infection, and cervical or vaginal ulcer, which has not been demonstrated in women previously. These findings could explain previous epidemiological observations indicating that both ulcerative and non-ulcerative STD are important risk factors for heterosexual transmission in Africa [8–12]. Of critical interest is the observation that among women who were cured of their STD, the proportion with HIV shedding decreased significantly, which has not been previously reported. These findings help to explain the facilitating role of STD for heterosexual HIV transmission, and are consistent with the recent important data from Mwanza, Tanzania, demonstrating that intensive treatment of STD can reduce HIV incidence [11]. Our data support the importance of genital ulcers as a facilitating factor for HIV transmission. When considered with data from earlier studies showing that genital ulcers are more frequent in immunosuppressed HIV-infected female sex workers [43,44], this study confirms the synergistic relationship between genital ulcers and HIV infection [45].

Vulvar ulcers were not associated with cervicovaginal shedding of HIV-1, as might have been expected, since because of their anatomical location they would not have been sampled by the lavage technique. The lack of association with trichomoniasis may be explained by a difference in the nature of the inflammatory exudate involved in cervical versus vaginal infections. The lack of association with syphilis may be explained by the absence of ulcers for the great majority of women with syphilis, which was defined serologically. Somewhat reassuringly and in contrast to previous studies, HIV-1 shedding was not found to be associated with use of oral contraceptives [23], with ectopy [23] or pregnancy [22,23], although all three were rare in this study.

We believe that PCR on cervicovaginal lavage fluid is a useful tool for assessing the presence of HIV in the female genital tract. Lavage sampling may better reflect the composition of cervicovaginal secretions than swabbing [23,24], since swabbing may provoke bleeding that could introduce HIV into the genital tract. A study of cervicovaginal HIV shedding in relation to the menstrual cycle also concluded that RNA PCR on the supernatant of a cervicovaginal lavage had a higher and more consistent yield than DNA PCR on cell pellets obtained from vaginal or cervical swabs [46].

In conclusion, these data deepen our understanding of a number of critical epidemiological observations on heterosexual HIV transmission, and lend biological plausibility to arguments for making STD control an integral part of HIV prevention strategies in Africa.

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References

1. Piot P, Quinn TC, Taelman H, *et al.*: **Acquired immunodeficiency syndrome in a heterosexual population in Zaïre.** *Lancet* 1984, **ii**:65–69.
2. Djomand G, Greenberg AE, Sassan-Morokro M, *et al.*: **The epidemic of HIV/AIDS in Abidjan, Côte d'Ivoire: a review of data collected by Projet RETRO-CI from 1987 to 1993.** *J Acquir Immune Defic Syndr* 1995, **10**:358–365.
3. Plummer FA, Nagelkerke NJD, Moses S, Ndinya-Achola JO, Bwayo J, Ngugi E: **The importance of core groups in the epidemiology and control of HIV-1 infection.** *AIDS* 1991, **5** (suppl 1):S169–S176.
4. Diallo MO, Ackah AN, Lafontaine M-F, *et al.*: **HIV-1 and HIV-2 infections in men attending sexually transmitted disease clinics in Abidjan, Côte d'Ivoire.** *AIDS* 1992, **6**:581–585.
5. Schutz R, Savarit D, Kadjo J-C, *et al.*: **Excluding blood donors at high risk of HIV infection in a west African city.** *BMJ* 1993, **307**:1517–1519.
6. Sassan-Morokro M, Greenberg AE, Coulibaly I-M, *et al.*: **High rates of sexual contact with female sex workers, sexually transmitted diseases, and condom neglect among HIV-infected and uninfected men with tuberculosis in Abidjan, Côte d'Ivoire.** *J Acquir Immune Defic Syndr* 1995, **11**:183–187.
7. De Vincenzi I, for the European Study Group on Heterosexual Transmission of HIV: **A longitudinal study of human immunodeficiency virus transmission by heterosexual partners.** *N Engl J Med* 1994, **331**:341–346.
8. Cameron DW, Simonsen JN, D'Costa LJ, *et al.*: **Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men.** *Lancet* 1989, **ii**:403–407.
9. Plummer FA, Simonsen JN, Cameron DW, *et al.*: **Cofactors in male–female sexual transmission of human immunodeficiency virus type 1.** *J Infect Dis* 1991, **163**:233–239.
10. Laga M, Alary M, Nzila N, *et al.*: **Condom promotion, sexually transmitted diseases treatment, and declining incidence of HIV-1 infection in female Zaïrian sex workers.** *Lancet* 1994, **344**:246–248.
11. Grosskurth H, Mosha F, Todd J, *et al.*: **Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial.** *Lancet* 1995, **346**:530–536.
12. Laga M, Diallo MO, Buvé A: **Inter-relationship of sexually transmitted diseases and HIV: where are we now?** *AIDS* 1994, **8** (suppl 1):S119–S124.
13. Kanki PJ, Travers KU, M'Boup S, *et al.*: **Slower heterosexual spread of HIV-2 than HIV-1.** *Lancet* 1994, **343**:943–946.
14. Anderson DJ, O'Brien TR, Politch JA, *et al.*: **Effects of disease stage and zidovudine therapy on the detection of human immunodeficiency virus type 1 in semen.** *JAMA* 1992, **267**:2769–2774.
15. Moss GB, Overbaugh J, Welch M, *et al.*: **Human immunodeficiency virus DNA in urethral secretions in men: association with gonococcal urethritis and CD4 cell depletion.** *J Infect Dis* 1995, **172**:1469–1474.
16. Krieger JN, Coombs RW, Collier AC, *et al.*: **Recovery of human immunodeficiency virus type 1 from semen: minimal impact of stage of infection and current antiviral chemotherapy.** *J Infect Dis* 1991, **163**:386–388.
17. Liuzzi G, Bagnarelli P, Chirianni A, *et al.*: **Quantitation of HIV-1 genome copy number in semen and saliva [letter].** *AIDS* 1995, **9**:651–653.
18. Hamed KA, Winters MA, Holodniy, Katzenstein DA, Merigan TC: **Detection of human immunodeficiency virus type 1 in semen: effects of disease stage and nucleoside therapy.** *J Infect Dis* 1993, **167**:798–802.
19. Atkins MC, Carlin EM, Emery VC, Griffiths PD, Boag F: **Fluctuations of HIV load in semen of HIV positive patients with newly acquired sexually transmitted diseases.** *BMJ* 1996, **313**:341–342.
20. Vogt MW, Craven DE, Crawford DF, *et al.*: **Isolation of HTLV-III/LAV from cervical secretions of women at risk for AIDS.** *Lancet* 1986, **i**:525–527.
21. Wofsy CB, Hauer LB, Michaelis BA, *et al.*: **Isolation of AIDS-associated retrovirus from genital secretions of women with antibodies to the virus.** *Lancet* 1986, **i**:527–529.
22. Hénin Y, Mandelbrot L, Henrion R, Pradinaud R, Coulaud JP, Montagnier L: **Virus excretion in the cervicovaginal secretions of pregnant and nonpregnant HIV-infected women.** *J Acquir Immune Defic Syndr* 1993, **6**:72–75.
23. Clemetson DA, Moss GB, Willerford DM, *et al.*: **Detection of HIV DNA in cervical and vaginal secretions.** *JAMA* 1993, **269**:2860–2864.

24. Kreiss J, Willerford DM, Hensel M, *et al.*: **Association between cervical inflammation and cervical shedding of human immunodeficiency virus DNA.** *J Infect Dis* 1994, **170**:1597–1601.
25. Ghys PD, Diallo MO, Ettiègne-Traoré V, *et al.*: **Dual seroreactivity to HIV-1 and HIV-2 in female sex workers in Abidjan, Côte d'Ivoire.** *AIDS* 1995, **9**:955–958.
26. Kalé K, Mah-bi G, N'da K, Tawil O, Ferencic N: **Prevention of STD/AIDS among prostitutes and clients in Abidjan: initial phase of implementation.** *IX International Conference on AIDS/IV STD World Congress.* Berlin, June 1993 [abstract PO-C14-2901].
27. Taylor-Robinson D: **Tests for infection with *C. trachomatis*.** *Int J STD AIDS* 1996, **7**:19–26.
28. Van Dyck E, Piot P, Meheus A: *Bench-Level Laboratory Manual for Sexually Transmitted Diseases.* Geneva: World Health Organization; 1989 [WHO/VDT/89.443].
29. Lockett AE, Dance DAB, Mabey DCW, Drasar BS: **Serum-free media for isolation of *Haemophilus ducreyi* [letter].** *Lancet* 1991, **338**:326.
30. Fransen K, Zhong P, De Beenhouwer H, *et al.*: **Design and evaluation of new, highly sensitive and specific primers for polymerase chain reaction detection of HIV-1 infected primary lymphocytes.** *Mol Cell Probes* 1994, **8**:317–322.
31. Alaeus A, Lidman K, Sönnerborg A, Albert J: **Subtype-specific problems with quantification of plasma HIV-1 RNA.** *AIDS* 1997, **11**:859–865.
32. Peeters M, Gershy-Damet G-M, Fransen K, *et al.*: **Virological and polymerase chain reaction studies of HIV-1/HIV-2 dual infection in Côte d'Ivoire.** *Lancet* 1992, **340**:339–340.
33. George JR, Ou C-Y, Bharat P, *et al.*: **Prevalence of HIV-1 and HIV-2 mixed infections in Côte d'Ivoire.** *Lancet* 1992, **340**:337–339.
34. Léonard G, Chaput A, Courgnaud V, Sangaré A, Denis F, Brechot C: **Characterization of dual HIV-1 and HIV-2 serological profiles by polymerase chain reaction.** *AIDS* 1993, **7**:1185–1189.
35. Simon F, Matheron S, Tamalet C, *et al.*: **Cellular and plasma viral load in patients infected with HIV-2.** *AIDS* 1993, **7**:1411–1417.
36. De Cock KM, Adjorlolo G, Ekpini E, *et al.*: **Epidemiology and transmission of HIV-2. Why there is no HIV-2 pandemic.** *JAMA* 1993, **270**:2083–2086.
37. Ariyoshi K, Berry N, Wilkins A, *et al.*: **A community-based study of human immunodeficiency virus type 2 provirus load in a rural village in West Africa.** *J Infect Dis* 1996, **173**:245–248.
38. Kunanusont C, Foy HM, Kreiss JK, *et al.*: **HIV-1 subtypes and male-to-female transmission in Thailand.** *Lancet* 1995, **345**:1078–1083.
39. Ellenberger D, Luo C-C, Pieniazek D, *et al.*: **Analysis of genetic diversity of HIV-1 in Côte d'Ivoire using restriction fragment length polymorphism (RFLP) analyses.** *XI International Conference on AIDS.* Vancouver, July 1996 [abstract TuA2065].
40. Laga M, Taelman H, Van der Stuyft P, Bonneux L, Vercauteren G, Piot P: **Advanced immunodeficiency as a risk factor for heterosexual transmission of HIV.** *AIDS* 1989, **3**:361–366.
41. Nielsen K, Boyer P, Dillon M, *et al.*: **Presence of human immunodeficiency virus (HIV) type 1 and HIV-1-specific antibodies in cervicovaginal secretions of infected mothers and in the gastric aspirates of their infants.** *J Infect Dis* 1996, **173**:1001–1004.
42. Rasheed S, Li Z, Xu D, Kovacs A: **Presence of cell-free human immunodeficiency virus in cervicovaginal secretions is independent of viral load in the blood of human immunodeficiency virus-infected women.** *Am J Obstet Gynecol* 1996, **175**:122–130.
43. Ghys PD, Diallo MO, Ettiègne-Traoré V, *et al.*: **Genital ulcers associated with human immunodeficiency virus-related immunosuppression in female sex workers in Abidjan, Ivory Coast.** *J Infect Dis* 1995, **172**:1371–1374.
44. Cameron DW, Ngugi EN, Ronald AR, *et al.*: **Condom use prevents genital ulcers in women working as prostitutes.** *Sex Transm Dis* 1991, **18**:188–191.
45. Wasserheit JN: **Epidemiological synergy. Interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases.** *Sex Transm Dis* 1992, **19**:61–77.
46. Goulston C, Stevens E, Gallo D, Mullins JI, Hanson CV, Katzenstein D: **Human immunodeficiency virus in plasma and genital secretions during the menstrual cycle.** *J Infect Dis* 1996, **174**:858–861.