

## CONCISE COMMUNICATION

## Human Immunodeficiency Virus Type 1 (HIV-1) Plasma Virus Load and Markers of Immune Activation among HIV-Infected Female Sex Workers with Sexually Transmitted Diseases in Abidjan, Côte d'Ivoire

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Plasma levels of human immunodeficiency virus type 1 (HIV-1) RNA and markers of immune activation were compared among HIV-1-infected female sex workers (FSWs) with ( $n = 112$ ) and without ( $n = 88$ ) sexually transmitted diseases (STDs) in Abidjan, Côte d'Ivoire. After adjustment for CD4<sup>+</sup> T cells, the median virus load was 2.5-fold higher among HIV-seropositive FSWs with STDs than among those without an STD ( $P = .053$ ). Median virus load was higher for FSWs with a genital ulcer ( $P = .052$ ) or gonorrhoea ( $P = .058$ ) than for FSWs without any STD. Median levels of markers of immune activation (CD38 and HLA-DR on CD8<sup>+</sup> T cells, soluble tumor necrosis factor- $\alpha$  receptor II, and  $\beta_2$ -microglobulin) tended to be elevated, albeit nonsignificantly, among FSWs in the STD group. These findings have important public health implications in elaborating strategies for decreasing disease progression and transmission of HIV among FSWs.

The explosive epidemic of human immunodeficiency virus type 1 (HIV-1) infection in Africa is characterized primarily by heterosexual transmission [1]. Reasons for this rapidly expanding epidemic may be multifactorial and may include epidemiologic and biologic factors. Epidemiologic studies have established clearly that sexually transmitted diseases (STDs) increase susceptibility to HIV infection [2] and vaginal shedding of HIV [3]. Thus, female sex workers (FSWs) may have a critical role in the HIV-1 epidemic in Africa, because of their high number of partners and their high rates of HIV and STDs [4].

We hypothesize that chronic cellular immune activation resulting from persistent infection with STDs in these women may be an important biologic factor that has a role in the increasing rate of HIV among FSWs. Indeed, immune activation of CD4<sup>+</sup> T cells is critical in HIV pathogenesis: after stimulation, the increased numbers of activated CD4<sup>+</sup> T cells serve as susceptible targets for HIV in vivo and for virus production, thereby increasing plasma levels of HIV-1 RNA [5]. Plasma virus load predicts the concentration of virus in vaginal fluid [6], correlates with heterosexual transmission of HIV-1 [7], and is a major risk factor for progression to AIDS and death [8]. Thus, cofactors that increase levels of the virus probably will facilitate HIV-1 transmission. Understanding the biologic factors that influence HIV-1 transmission may be essential for managing and controlling HIV infection in Africa. The objective of this study was to compare the plasma levels of HIV-1 RNA and markers of immune activation among STD-infected and -uninfected FSWs enrolled at a confidential clinic in Abidjan, Côte d'Ivoire.

### Population and Methods

**Study population.** This cross-sectional study was conducted from September 1996 to June 1997 as part of a larger study that assessed the associations among cervicovaginal HIV shedding, STDs, and immunosuppression among consenting FSWs consecutively enrolled at a confidential clinic in Abidjan [3]. For our study, we included those FSWs who were seropositive for HIV-1 or dually

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The study was approved by the ethical committee of the Côte d'Ivoire Ministry of Health, the institutional review boards of the Centers for Disease Control and Prevention, Atlanta, and the Institute of Tropical Medicine, Antwerp, Belgium. Research followed the experimentation guidelines of the US Department of Health and Human Services and those of the authors' institutions.

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seropositive for HIV-1 and HIV-2 and who had available information on CD4<sup>+</sup> T cell counts, STDs, or both.

**Laboratory methods.** Whole blood from the FSWs was collected into Vacutainer tubes (Becton Dickinson) containing sodium citrate gel and density gradient media. Within 6 h, plasma was separated from cells by centrifugation at 200 g, was aliquoted, and was stored at -70°C.

**HIV and STD diagnosis.** HIV-1 antibody status and HIV type-specific serodiagnosis were determined by use of an ELISA-based strategy [9]. Laboratory or clinical methods were used to detect STDs: antitreponemal antibodies were detected, using *Treponema pallidum* hemagglutination assay (TPHA; Fujirebio) and rapid plasma reagin (RPR; Macro-Vue; Becton Dickinson). A diagnosis of syphilis was made if results for both the TPHA and RPR tests were positive [3]. *Neisseria gonorrhoeae* infection was diagnosed by culture on modified Thayer-Martin medium [3]. *Trichomonas vaginalis* infection was diagnosed by use of wet mount techniques, and *Chlamydia trachomatis* was diagnosed by EIA (Syva). Genital ulcers were diagnosed clinically by visualization, with an appropriate light source, of the external genital area, the vulva, and the vagina and cervix, after the placement of a speculum.

**HIV-1 RNA virus load.** Plasma RNA virus load was quantified in plasma by use of the Amplicor HIV-1 Monitor test (version 1.5; Roche Diagnostics Systems). The lower limit of detection of this assay is 200 copies/mL. All assays were done as recommended by the manufacturers.

**Immunologic subsets and activation markers.** CD4<sup>+</sup> and CD8<sup>+</sup> T cells and markers of immune activation (CD25, CD38, HLA-DR, and CD28) were analyzed, using 3-color flow cytometry (FAC-Scan; Becton Dickinson), on fresh peripheral whole blood. CD25, which is expressed predominantly on CD4<sup>+</sup> T cells, decreases in HIV patients, possibly as a consequence of chronic immune activation. CD38 is re-expressed on primed cells after activation in HIV-infected persons, and its expression on CD8<sup>+</sup> T cells increases significantly with disease progression. HLA-DR is a class II antigen that is expressed on activated T cells. CD28 is an accessory molecule that is expressed on T cells but is lost after chronic activation. Soluble tumor necrosis factor- $\alpha$  receptor II (sTNF- $\alpha$ R2) and  $\beta_2$ -microglobulin were measured in plasma specimens by use of commercial ELISAs (Quantikine; R&D Systems).

**Statistical analysis.** A simple comparison of 2 groups was done using the nonparametric Mann-Whitney *U* test for unpaired data. Categorical variables were compared by  $\chi^2$  analysis. The association of STDs and virus load was calculated after adjusting for differences in CD4<sup>+</sup> T cell counts and HIV serostatus. This was done by entering all STDs simultaneously in a multiple linear regression model after adjustments for CD4<sup>+</sup> T cell counts (continuous variable) and HIV serostatus had been entered into the model. The study had 80% power at 5% level to detect differences in virus load >0.5 log<sub>10</sub> copies/mL among FSWs with and without STDs, with a within-patient SD of 1.08 log<sub>10</sub> copies/mL virus load. Thus, a sample size of 68 in each group of FSWs would have been sufficient to achieve this power. The nonparametric Spearman's rank correlation coefficient was used to assess correlations.

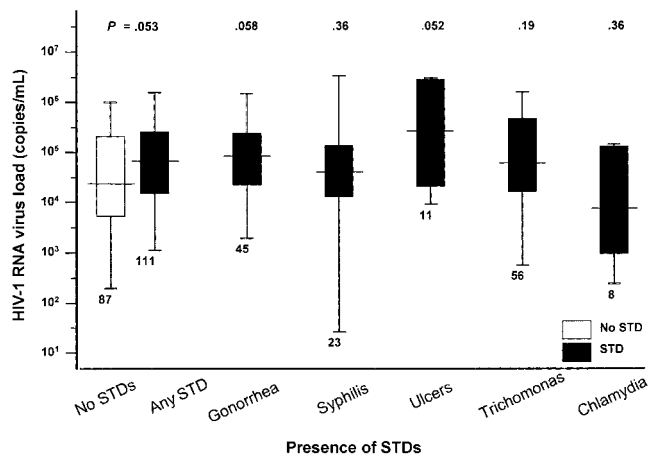
## Results

**Characteristics of the study population.** Of the 200 FSWs who were eligible for enrollment in this study, 112 (56%) had  $\geq 1$

STD. The distribution of individual STDs among the FSWs was as follows: trichomoniasis, 56 (28%); *Neisseria gonorrhoeae*, 45 (23%); syphilis, 24 (12%); genital ulcers, 11 (5.5%); and chlamydia infection, 8 (4%). The median age of the FSWs with or without STDs was 27 years. The median duration of their sex work career was 24 months (interquartile range [IQR], 11–48 months) among FSWs with STDs and 36 months (IQR, 12–60 months) for those without an STD ( $P = .25$ ). Among the FSWs with available CD4<sup>+</sup> T cell counts, 162 had a median count of 510 cells/ $\mu$ L (IQR, 355–781 cells/ $\mu$ L); of these, 16 (9.9%) had <200 cells/ $\mu$ L, 64 (39.5%) had 200–499 cells/ $\mu$ L, and 82 (50.6%) had >499 cells/ $\mu$ L. The median age of these FSWs was 27 years (IQR, 21–32 years), and the duration of their sex work career was 36 months (IQR, 12–51 months). Median CD4<sup>+</sup> T cell counts were lower among FSWs with >1 STD (477 cells/ $\mu$ L; IQR, 335–750 cells/ $\mu$ L) than among those without an STD (525 cells/ $\mu$ L; IQR, 363–866 cells/ $\mu$ L;  $P = .14$ ). The median virus load, as determined on the basis of CD4<sup>+</sup> T cell count strata, was 5.6 log<sub>10</sub> copies/mL among FSWs with a CD4<sup>+</sup> T cell count <200 cells/ $\mu$ L, 5.2 log<sub>10</sub> copies/mL among those with 200–499 cells/ $\mu$ L, and 4.2 log<sub>10</sub> copies/mL among those with >500 cells/ $\mu$ L. There was a significant inverse correlation between plasma HIV-1 RNA loads and CD4<sup>+</sup> T cell counts ( $r = -0.51$ ;  $P < .001$ ).

**Association of STDs with virus load.** Because CD4<sup>+</sup> T cell counts inversely correlate significantly with virus load, we entered CD4<sup>+</sup> T cell counts in the analysis as a covariate, to assess the STD factor independently from CD4<sup>+</sup> T cell counts. Taking together data for all the STDs, the median plasma HIV-1 RNA load was 2.5-fold higher among the HIV-1-seropositive FSWs with an STD (median, 4.9 log<sub>10</sub> copies/mL; IQR, 4.2–5.4 log<sub>10</sub> copies/mL) than that among FSWs without an STD (median, 4.4 log<sub>10</sub> copies/mL; IQR, 3.7–5.3 log<sub>10</sub> copies/mL;  $P = .053$ ; figure 1). When we considered the effect of individual STDs on virus load, only the presence of genital ulcers and gonococcal infection were associated with higher virus load when adjusted for CD4<sup>+</sup> T cell counts ( $P = .058$  and  $P = .052$ , respectively). Virus load also was slightly higher among FSWs diagnosed with syphilis and trichomonas infection than among FSWs without an STD (figure 1).

**Association of STDs with inflammatory cytokines and immune activation markers.** Activated CD4<sup>+</sup> and CD25<sup>+</sup> CD4 T cells are preferential target cells for HIV infections. In addition, increased activation of CD8 cells in HIV-infected persons is associated with disease progression and poorer prognosis. Thus, we reasoned that infection with STDs may increase chronic immune activation of CD8<sup>+</sup> T cells and may enhance viral replication of HIV and accelerate disease progression. In this study, which focused on HIV-1 seropositive FSWs, FSWs with STDs tended to have higher median levels of immune activation, albeit insignificantly, than did those without STDs. For instance, the median expression of HLA-DR<sup>+</sup> on CD4<sup>+</sup> T cells (target cells), CD38 on CD8<sup>+</sup> T cells, and HLA-DR on CD8<sup>+</sup> T cells was higher. Levels of expression of CD25 on CD4<sup>+</sup> T



**Figure 1.** Comparison of plasma concentrations of human immunodeficiency virus type 1 (HIV-1) RNA in HIV-seropositive female sex workers (FSWs) with sexually transmitted diseases (STDs) and those without STDs. Data are median and interquartile range; the 5%–95% data range is represented by error bars. Nos. below the bars represent FSWs with and without STDs.

cells and CD28 on CD4<sup>+</sup> T cells that are known to decrease after activation [10] were lower, but not significantly, among FSWs with STDs (figure 2). Median levels of sTNF- $\alpha$ RII and  $\beta_2$ -microglobulin were higher, but not significantly, among FSWs with STDs than among those without STDs (figure 2).

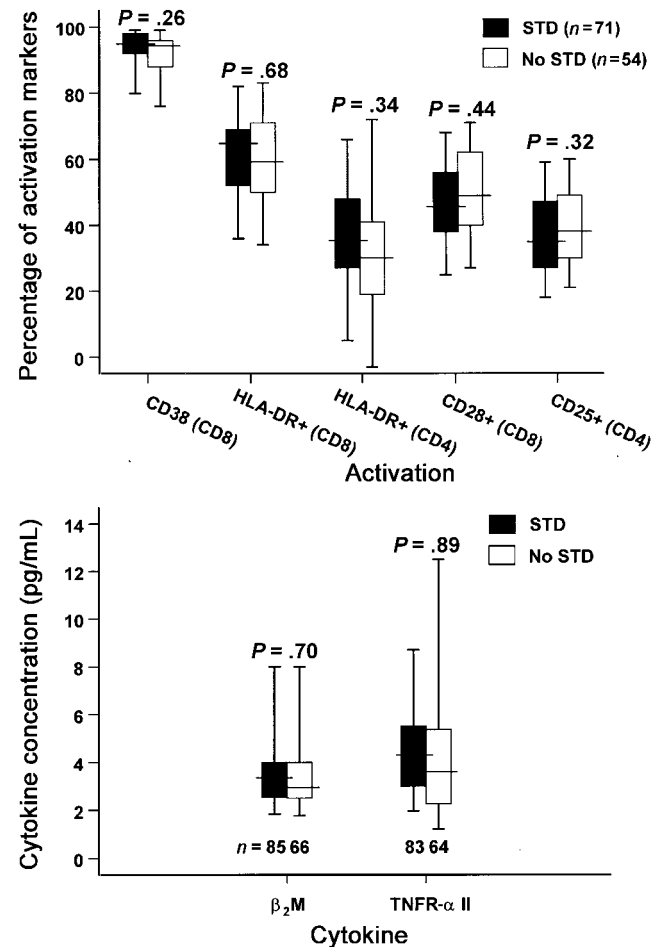
**Discussion**

A major finding from our study is that, after we adjusted for differences in CD4<sup>+</sup> T cell counts, the plasma HIV-1 load was 2.5-fold higher among HIV-infected FSWs with STDs than among FSWs without STDs. This increase is consistent with virus load increases caused by other infectious agents in HIV-infected persons [11, 12]. Our results are consistent with a recent study among FSWs in Nairobi that found increased plasma levels of HIV-1 RNA and increased levels of cytokines during STD infections, especially among subjects with gonococcal infection [13]. Other studies in Malawi found higher levels of HIV-1 load in seminal, but not blood, plasma among subjects with gonorrhoea and its attendant inflammation [14].

Increased activation of CD4<sup>+</sup> T cells and proinflammatory cytokines are thought to have a critical role in the pathogenesis of HIV infection, because >95% of free virus in blood comes from activated CD4<sup>+</sup> T cells [15]. Our results show a tendency, although not statistically significant, for increased immune activation among HIV-seropositive FSWs with STDs (figure 2) that may partly explain the increased virus load among FSWs with STDs.

Because FSWs probably have an important role in the epidemic of HIV in Africa, we believe that our findings of elevated plasma HIV-1 load in FSWs with STDs may have several im-

portant implications. First, increase in plasma HIV-1 load now is recognized as a major risk factor for progression to AIDS and death [8]. Second, our finding may have implications for heterosexual transmission of HIV from STD-infected FSWs to their clients. A Ugandan study showed that no heterosexual transmission of HIV-1 occurred when virus load was <1500 copies/mL; however, each log<sub>10</sub> incremental increase in virus load was associated with a 2.5-fold increased risk for heterosexual transmission of HIV-1 [7]. Thus, although in our study virus loads were >1500 copies/mL in FSWs with and those without STDs, factors that contribute to elevated virus load may increase the risk for HIV-1 heterosexual transmission. In addition, plasma RNA virus load correlates with cervicovaginal HIV-1 RNA levels [6]. Identifying and decreasing HIV transmission risk among core transmitters, such as FSWs, who play



**Figure 2.** Comparison of cellular immune activation markers and proinflammatory cytokine levels in human immunodeficiency virus (HIV)-seropositive female sex workers with sexually transmitted diseases (STDs) and those without STDs. Data are median and interquartile range; the 5%–95% data range is represented by error bars.  $\beta_2$ M,  $\beta_2$ -microglobulin; TNFR- $\alpha$ II, tumor necrosis factor- $\alpha$  receptor II.

a key role in emerging epidemic, may be cost-effective in halting the HIV epidemic.

Our study is limited by its cross-sectional design; for instance, the association observed between the presence of genital ulcer and HIV-1 load may be due to the presence of genital herpes, which tends to recur frequently during advanced HIV infection. Thus, there is a need for longitudinal studies in which plasma virus load and immunologic parameters are monitored in FSWs before and after treatment for STDs.

In summary, we have shown that, in Abidjan, plasma virus load is higher among HIV-1-infected FSWs with STDs than among those without an STD. In addition, immune activation and proinflammatory markers tended to be higher also, albeit insignificantly, in FSWs with STDs. Our findings have important public health implications in the elaboration of strategies for decreasing disease progression and transmission of HIV among FSWs.

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