Sex Differences in Longitudinal Human Immunodeficiency Virus Type 1 RNA Levels among Seroconverters

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Cross-sectional studies have demonstrated lower plasma human immunodeficiency virus type 1 (HIV-1) RNA virus levels (VLs) in women than in men, but it is unknown whether this sex difference is present at the time of seroconversion and throughout the course of infection. A nested case-control study was performed among HIV-1 seroconverters within a cohort of injection drug users. Plasma VL was determined longitudinally among both rapid progressors to AIDS (24 patients) and nonprogressors (47 controls). The initial median VL among female patients (n = 10) was 14,918 copies/mL, compared with 148,354 copies/mL among male patients (n = 14; P = .001); median plasma VL also tended to be lower among female (n = 10) than among male controls (n = 37; 11,917 vs. 61,311 copies/mL; P = .08). VL increased more rapidly over time in women than in men and subsequently converged in patients and controls, respectively. Understanding the mechanisms responsible for the sex difference in VL may provide insight into HIV-1 pathogenesis.

Plasma human immunodeficiency virus type 1 (HIV-1) RNA virus level (VL) after acute HIV-1 seroconversion is an independent predictor of progression to AIDS [1–5]. The risk of progression to AIDS, according to VL, has been shown among homosexual men [6, 7], hemophiliacs [8], and injection drug users (IDUs) [9]. Many of these studies helped form the basis for current recommendations in regard to the plasma VL at which antiretroviral therapy should be initiated [10, 11]. A recent study among homosexual men related longitudinal changes in plasma VL to disease progression and noted that annual plasma VL was more predictive of progression to AIDS than the initial VL [12]. A longitudinal comparison of VL from the time of seroconversion in both women and men has not been done, however.

Cross-sectional [13, 14] and longitudinal [15] studies have reported lower HIV-1 RNA levels in women than in men after controlling for CD4 cell count, with no difference observed in the time to the development of AIDS. In a small study among seroprevalent persons, median plasma VL was lower in women than in men [16], but the difference was not statistically significant; in another study, no difference in plasma VL between women and men was found [17]. Because all of these studies were done in seroprevalent cohorts, they were limited by the possible bias that can occur when the onset of infection is unknown. We therefore performed a nested case-control study, to assess the patterns of plasma VL over time among rapid progressors and nonprogressors from the time of seroconversion within a cohort study of HIV-1 infection in male and female IDUs. The nested case-control design enabled identification of rapid and nonrapid progressors, in whom trends in VL could then be determined from previously collected specimens.

Methods

Study Population

Between February 1988 and March 1989, 2960 IDUs from Baltimore were enrolled in a longitudinal study of HIV-1 infection among IDUs [18]. Criteria for study entry were age ≥18 years, injection of drugs at least once after 1977, and AIDS-free status. Of the 2960 IDUs who were enrolled, 704 were HIV seropositive and 2256 were HIV seronegative. Between March 1989 and July 1996, there were 272 HIV-1 seroconverters.

Data Collection

All HIV-1-seropositive and a sample of seronegative participants underwent a physical examination and had blood drawn semian-
ually for laboratory studies; additional blood was frozen for future studies. Information on demographics, injection drug use, sexual practices, medical history, and use of medications (including antiretroviral therapy for HIV-seropositive participants) was obtained semiannually. Trained nurses abstracted medical records by use of standard forms, and an end-points committee led by a physician established final diagnoses. AIDS-defining diagnoses were determined according to the 1993 CDC clinical case definition [19] (excluding CD4 cell count <200 lymphocytes/mm³), based upon the review of medical records and death certificates received through 30 June 1997.

**Nested Case-Control Selection**

Both patients and controls came from the AIDS Linked to the Intravenous Experience (ALIVE) cohort. Only participants who became seropositive before 1 July 1996 and for whom the time between the last negative and first positive HIV-1 antibody titer was ≤12 months were eligible for selection. Among the 272 documented cases of seroconversion through July 1996, 215 were eligible for selection in the case-control study.

**HIV-1 seroconverters with AIDS (case group).** The case set was defined as eligible HIV-1 seroconverters who met the following 3 conditions: (1) AIDS diagnosis before 1 July 1996, (2) ≥3 follow-up visits after the estimated seroconversion date but before the AIDS diagnosis date, and (3) ≥3 plasma specimens after the estimated seroconversion date but before the AIDS diagnosis date available in the repository for virus load quantification. Of the 215 eligible HIV-1 seroconverters, 31 developed AIDS before 1 July 1996; 3 did not satisfy criterion 2 and 3 did not satisfy criterion 3, resulting in 25 cases. Of the 6 patients who were excluded, 2 were male and 4 were female.

**AIDS-free HIV-1 seroconverters (control group).** The pool of controls was defined as eligible HIV-1 seroconverters who had no documented AIDS diagnosis before 1 July 1996, ≥3 follow-up visits and plasma HIV-1 RNA determinations after the estimated seroconversion date but before 1 July 1996. Of the 184 AIDS-free subjects, there were 156 possible controls for matching.

**Matching.** The closest 2 controls were selected for each patient, based on the following ranked matching criteria: (1) age at the estimated seroconversion date (±5 years of the patient), (2) estimated seroconversion date (within 6 months of the patient), and (3) follow-up time from estimated seroconversion date to last visit prior to 1 July 1996, as least as long as that of the patient from the estimated seroconversion date to the AIDS diagnosis date. No match was found for 1 patient, and 1 patient could be matched to only 1 control. Only 1 control was excluded, and therefore replaced, because of a lack of repository specimens. The matching procedure therefore resulted in 24 matched case sets: 23 patients with 2 matched controls and 1 patient with 1 matched control for a total of 24 cases and 47 controls.

**Laboratory Techniques**

Antibodies to HIV-1 were measured by commercial ELISA kit (Genetic Systems, Seattle), and positive specimens were confirmed by Western blot (DuPont, Wilmington, DE). T cell subsets were measured by modified whole blood staining methods and flow cytometry [20, 21]. Heparinized plasma specimens for quantification of virus load were stored at −70°C until testing. Levels of HIV-1 RNA in plasma were quantified by reverse transcriptase polymerase chain reaction (PCR) assay (Roche Molecular Systems, Branchburg, NJ). Viral RNA was extracted by a silica extraction method [22], and the assay was then performed according to the manufacturer’s protocol for thawed plasma. The minimum detectable level of HIV-1 RNA was 400 copies/mL, and the dynamic range of the assay was −4 logs. Undetectable VL determinations were censored as one-half of the detectable limit (i.e., 200 copies/mL). Plasma VL quantification was performed for HIV seroconverters at all study visits, up to and including the first visit after an AIDS diagnosis for patients or until 1 July 1996 for controls.

**Statistical Analysis**

The Wilcoxon rank sum test was used to compare continuous variables between groups. The χ² test was used to compare categorical variables; Fisher’s two-tailed exact test was used when the sample size was small. Locally weighted regression smoothing was used to produce Loess plots of VL trajectories over time, where time was measured as years since seroconversion. The seroconversion date was estimated as the midpoint between the last negative visit date and the first positive visit date. Random effect models were used to model the patterns of log-transformed plasma VL over time, while accounting for the correlation between repeated VL measures within a subject [23]. The VL data were censored at the first visit date after AIDS diagnosis for patients and at 1 July 1996 for controls. After considering 3 correlation structures for the repeated data, the spatial correlation structure was considered most appropriate and was used for the analysis by patient status and sex. A separate random effect of within-class correlation for the case set, to account for the matched-pair design, was assessed and did not contribute to the inferences. Differences in virus levels and slopes by patient status and sex were evaluated via the Wald-based test statistic.

**Results**

**Viral Load after Seroconversion**

Comparison of patients and controls. There were 24 patients and 47 matched controls; demographic data are summarized in table 1. There were 179 participant visits among patients and 445 visits among controls, for a total of 624 visits. Plasma samples were available for 617 of these visits. There were, on average, 8.7 measures per subject (range, 3–16); 7.3 among patients and 9.4 among controls. No significant differences were noted between patients and controls in regard to ethnicity (predominantly black), current injection drug use, median age at seroconversion, or median time of first VL determination after seroconversion. The median year of seroconversion was 1990 for patients and 1989 for controls (P = .98). Sex composition, however, tended to differ by case-control status: 42% of patients were female, compared with 21% of controls (P = .07). In the full ALIVE cohort through 1 July 1996, 23% of all participants,
23% of seroconverters, and 26% of AIDS patients were female. Among the 7 patients (4 women, 3 men) excluded from this study, the median CD4 cell count at seroconversion was 464 lymphocytes/mm³ and the median time to AIDS was 28.6 months.

For the 611 visits for which such data were available (data missing for 6 visits), at 513 visits (84%) participants reported antiretroviral therapy use. Nucleoside monotherapy was reported at 15.4% of all visits, and combination nucleoside therapy was reported at 0.7% of visits. No subject in the sample population received protease inhibitors, since the study period ended in 1996. Only 1 subject received antiretroviral therapy during the 6 months prior to the first viral load measurement (a female control). Based on the mean percentage of visits per participant in which antiretroviral therapy use was reported, patients were no more likely than controls to have received antiretroviral therapy (17% vs. 16%; P = .94).

At the first seropositive visit, median plasma VL was higher in patients than in controls, but this difference was not statistically significant (table 1). In a multiple linear regression analysis adjusted for sex, time since seroconversion, and CD4 cell count, the mean plasma VL in men was 0.78 log (6-fold) higher than in women (P < .001). Median VL was higher among male patients than in male controls (148,354 vs. 61,311 copies/mL; P = .08). In a multiple linear regression analysis adjusting for case-control status, time since seroconversion, and CD4 cell count, the mean plasma VL in men was 0.78 log (6-fold) higher than in women (P < .001).

Median VL was higher among male patients than in male controls (148,354 vs. 61,311 copies/mL; P = .08), but not among female patients compared with female controls (14,918 vs. 11,971 copies/mL; P = .68). In a multiple linear regression analysis among only women, after adjusting for CD4 cell count and time since seroconversion, initial median plasma VL was 0.45 log higher in patients than in controls, but this difference was not statistically significant (P = .30).

### Longitudinal Follow-up of Viral Load

**Comparison of patients and controls.** Figure 1 shows the median levels and interquartile ranges for the plasma VL and CD4 cell counts longitudinally after estimated seroconversion by case-control status. On the basis of observation, it appears that median plasma VL increased over time until AIDS diagnosis among patients, but decreased and subsequently stabilized among controls. The decline in CD4 cell count was greater in patients than in controls. On average, VL was more than one-half log higher among patients than among controls (P = .0003), but the difference changed significantly over time. For example, 6 months after seroconversion, while controlling for sex, VL was an estimated 0.45 log higher (2.8-fold increase) in patients versus controls (P = .92). This difference increased by 0.11 log annually (P = .06), such that VL was an estimated 0.83 log higher (6.8-fold increase) among patients within 4 years after seroconversion.

Because of the decrease in the number of VL determinations as more patients developed AIDS, we assessed trends in VL over the first 3 years after seroconversion. Differences in VL between patients and controls and in slopes over time were similar to those during the entire study period, although statistical significance was not attained because of fewer data points.
Differences between men and women. The increase in VL over time among female patients was significantly greater than the increase among male patients (0.24 log VL/year for women vs. 0.003 log VL/year for men; \( P = .002 \); figure 2). Thus, despite a significantly lower median VL after seroconversion in women, median viral loads were equivalent in both women and men within 5 years after seroconversion. The rate of increase in VL over time was also greater in female than in male controls (0.087 log VL/year for women vs. –0.057 log VL/year for men; \( P = .04 \)); VL trajectories of female and male controls tended to converge within 7 years after seroconversion.

On average, women had greater than a quarter log (47%) lower VL than men (\( P = .09 \)), although this difference varied significantly over time. For example, 6 months after seroconversion, after controlling for case-control status, women had a 0.87 log (86% decrease) lower virus load than men (\( P = .0001 \)). This difference diminished by 0.16 log every year after seroconversion (\( P = .002 \)), and VL trajectories crossed an estimated 5.8 years after seroconversion. The sex effect on VL was similar among patients and controls, when stratified by case status (data not shown). Sex differences in VL slopes were similar over the first 3 years after seroconversion, compared with the entire study period. Antiretroviral therapy use did not alter the effect of case-control status or sex on VL. Although initial median VL did not differ significantly between women who did or did not progress to AIDS, VL was significantly higher in female patients than in controls within 2 years after seroconversion (\( P = .004 \)).

Discussion

In previous studies of HIV-1 seroprevalent cohorts in which the date of onset of HIV infection was unknown, plasma HIV-1 RNA levels were lower in women than in men, after adjusting for CD4 cell count [13–15]. One possible methodologic explanation for this finding may be that women had been infected later in calendar time than men because of a more recent onset of the HIV-1 epidemic in women. We sought to address this issue by directly comparing HIV-1 RNA levels among women and men longitudinally from the time of seroconversion.

Among HIV-1 seroconverters in this study who subsequently progressed to AIDS, median VL after seroconversion was lower in women than in men, after controlling for age at seroconversion and CD4 cell count. Among the seroconverters who
did not progress to AIDS during the study period, median VL also tended to be lower in women than in men. Of interest, when trends in plasma VL were assessed over time, viral dynamics differed by sex. Despite lower initial VL in both female patients and controls than in their male counterparts, VL increased more rapidly over time in women than men. This was true in both patients and controls, such that VL trajectories converged within 5 years among patients and 7 years among controls. This finding is consistent with a previous study, in which VL was lower in women than in men cross-sectionally and in which the difference diminished over time [15]. This is also consistent with a report in which women had higher CD4 cell counts at presentation than men but more rapid decline over time [24].

Consistent with previous studies in men [1–5], plasma HIV-1 RNA level at seroconversion was associated with progression to AIDS among men in this study. Initial median plasma VL among female seroconverters was not significantly higher in patients than in controls, however, suggesting that initial VL in women in this study did not predict rapid progression to AIDS. This lack of a statistically significant difference in plasma VL could be due to the low number of women in this study. It was notable that the VL in female patients after seroconversion was significantly lower than the VL in male patients. Longitudinal follow-up revealed that plasma VL predicted rapid disease progression throughout the course of HIV-1 infection in men but was predictive in women only 2 years after seroconversion. The possible sex difference in the ability to predict disease progression based on initial VL warrants further study among larger populations.

Plasma VL was lower in women than in men among both progressors (patients) and nonprogressors (controls), suggesting differing virus-host interactions according to sex, not simply due to clinical disease status. The difference appeared to persist longer in nonprogressors than in progressors, all of whom were rapid (median time to AIDS, <5 years). The explanation for the diminishing difference in plasma VL over time is unclear. Because the sex difference in plasma VL was greatest early in

**Figure 2.** Loess-smoothed regression lines of longitudinal human immunodeficiency virus type 1 (HIV-1) level by sex and case status. Individual data points and regression lines are shown (see key on figure).
the course of infection, however, further study of these differences would most likely be best performed early after onset of infection.

There is some prior evidence of sex differences in the immune response to viral infections. Among a small seroprevalent cohort of hepatitis C virus (HCV) antibody–positive IDUs, women were significantly more likely to have undetectable HCV RNA by PCR in their serum than men, suggesting more effective virus clearance in women [25]. HCV burden was also significantly lower in women in a longitudinal study of IDUs [26]. In studies of hepatitis A [27] and hepatitis B [28] vaccination, a more efficient immune response was noted in women than in men. The humoral immune response to live measles vaccine is also greater in women than in men [29, 30]. In addition, in a study of natural rubella infection, antibody responses differed in women and in men (envelope E2–specific IgA antibody was produced in women but not in men) [31].

The mechanism underlying the sex difference in plasma virus load is unknown, but there are several possible hypotheses. Hormonal differences could be responsible. Estrogen down-regulates expression of tumor necrosis factor-α [32], which in turn directly affects HIV-1 expression [33]. The more pronounced cell-mediated and humoral immune response in women, which is possibly hormone related [34], could affect the cytotoxic T cell response, which appears to control HIV-1 replication and to slow disease progression [35]. A second hypothesis relates to the rapid rate of CD4 cell turnover in HIV-1 infection [36]. Sex differences in factors responsible for CD4 cell turnover (e.g., target cell infection, viral replication, and rate of CD4 cell killing) could result in less release of HIV-1 into plasma in women, resulting in lower VL but similar CD4 cell counts and similar time to AIDS. A third possible explanation could be that women are more likely to become HIV-infected through heterosexual exposure rather than via injection drug use and thus possibly receive a smaller inoculum of HIV-1. Women and men were equally likely to report recent injection drug use at the study visit prior to seroconversion, however, and it is unknown whether the inoculum via heterosexual transmission is smaller than that via injection drug use.

That women represented a greater proportion of patients (i.e., developed AIDS) than men despite lower initial VL and similar use of antiretroviral therapy is noteworthy. The over-representation of women among patients in this study could have been due to sampling variation in the cohort, but patients and controls were both representative of the ALIVE cohort according to race, current injection drug use, and age and CD4 cell count at seroconversion. Of the 7 AIDS patients from the cohort excluded from this study, 4 were women and 3 were men, indicating that case selection was representative of AIDS cases within the ALIVE cohort. Another possible explanation could be better follow-up of women than men, resulting in better ascertainment of AIDS-indicator diagnoses than in men.

Sex differences in AIDS-defining diagnoses could also contribute, but such differences were not found in this study.

Several study limitations should be acknowledged. First, sample size was small. Although this usually decreases the likelihood of detecting statistically significant differences, the presence of outliers could have exaggerated sex differences in VL. Small sample size also precluded an assessment of potential confounding factors on plasma VL. Second, because the ALIVE cohort is 100% IDU and >95% black, generalizability could be limited, although injection drug use [37] and race [9] have not been found to affect the natural history of HIV-1 infection. Third, follow-up time was limited to a maximum of 7 years, and the median time to AIDS among patients was relatively short (51 months). Because VL dynamics may differ among rapid progressors from those who develop AIDS more slowly, extrapolation of sex differences in VL trajectories over longer periods should be considered cautiously. Fourth, some of the controls could have developed AIDS by the end of the study but not have been identified because of losses to follow-up. Although such misclassification is unlikely, if present, it would diminish rather than increase any sex difference in VL. Replication of the findings in this study among other populations is therefore necessary.

With the above caveats acknowledged, our findings are consistent with earlier studies that identified lower plasma VL in women than in men, but they also establish that these differences exist at the time of seroconversion and persist for ≥4 years after seroconversion. Most striking was that the viral dynamics of HIV-1 infection differed among women and men over the course of infection. Understanding the mechanisms responsible for this difference may provide greater insight into HIV-1 pathogenesis. Although further studies are necessary to validate our findings, they suggest that antiretroviral therapy may need to be initiated at a lower virus level in women than in men.

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References


