Brief but Efficient: Acute HIV Infection and the Sexual Transmission of HIV

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Background. We examined whether viral dynamics in the genital tract during the natural history of acute human immunodeficiency virus type 1 (HIV-1) infection could explain efficient heterosexual transmission of HIV.

Methods. We measured HIV-1 concentration in blood and semen samples from patients with acute and long-term HIV-1 infection. We explored the effect of changes in viral dynamics in semen on the probability of transmission per coital act, using a probabilistic model published elsewhere.

Results. Considered over time from infection, semen HIV-1 concentrations, in men with acute infection, increase and decrease in approximate parallel with changes occurring in blood. Modeling suggests that these acute dynamics alone are sufficient to increase probability of heterosexual transmission by 8–10-fold between peak (day 20 after infection, based on the model) and virologic set points (day 54 and later after infection). Depending on the frequency of coitus, men with average semen HIV-1 loads and without sexually transmitted diseases (STDs) would be expected to infect 7%–24% of susceptible female sex partners during the first 2 months of infection. The predicted infection rate would be much higher when either partner has an STD.

Conclusions. Empirical biological data strongly support the hypothesis that sexual transmission by acutely infected individuals has a disproportionate effect on the spread of HIV-1 infection. Acute hyperinfectiousness may, in part, explain the current pandemic in heterosexual individuals.

The average probability of male-female transmission of HIV-1 per unprotected coital act has been estimated, in a large number of observational studies (reviewed in [1, 2]), to be .0005–.0026 (1/2000–1 transmission event/384 coital acts) during established (i.e., nonacute) HIV-1 infection. In a study using survey-based data on sexual behaviors in the United States, Pinkerton et al. [3] calculated that these probabilities of transmission per coital act would result in low rates of lifetime transmission (0.19–0.40 infected partners/man; 0.09–0.18 infected partners/woman), which, by themselves, could not sustain an epidemic. These estimates have been recently cited as evidence for the putative importance of iatrogenic spread in areas where the pandemic is growing the fastest [4]. However, most estimates of probability of transmission per coital act used for such calculations do not take into consideration biological factors that might increase or decrease the probability of transmission of HIV-1. Because the studies from which probabilities of transmission are derived enroll only HIV-1–discordant couples, it is important to note that the overall estimates generated from such studies generally reflect transmission by individuals with long-term infection—necessarily underestimating the potential influence of transmission by acutely infected individuals with peak HIV-1 loads [5]. Transiently high viremia could translate to heightened probability of transmission during acute infection. Indeed, in a recent study of HIV-1–serodiscordant couples in Uganda,
Gray et al. [2] observed a strong relationship between blood HIV-1 load and probability of heterosexual transmission.

However, that genital fluids (not blood) are the principal vehicle for sexual transmission of HIV presents a particular problem for modeling the likelihood of HIV transmission during acute HIV-1 infection on the basis of blood data. This is because acute HIV-1 infection represents the period of initial establishment of anatomic HIV-1 reservoirs; therefore, the viral dynamics in blood, which have been well described for acute HIV-1 infection [6–9], cannot be assumed to apply to the genital tract. If HIV-1 load were to increase more rapidly in genital fluids than in the systemic compartment, for instance, the probability of transmission during acute HIV-1 infection would be greater than that predicted on the basis of concurrent blood HIV-1 load; if, on the other hand, semen HIV-1 load were to increase slowly, relative to blood HIV-1 load, it is possible that no peak in probability of transmission would occur at all, despite elevated blood HIV-1 load during acute HIV-1 infection. Previous human studies examining the excretion of HIV-1 in semen during acute HIV-1 infection have been limited by an inability to obtain semen samples over time from untreated patients with acute HIV-1 infection. In a study involving experimental infection of macaques with HIV type 2GB122 or simian/HIV 

PATIENTS, MATERIALS, AND METHODS

Data on Patients

Patients with acute HIV-1 infection. For preliminary descriptive modeling of compartmental viral dynamics, both blood and semen HIV-1 concentrations were available for men with known dates of HIV-1 infection or with known dates of onset of an acute retroviral syndrome, who had been enrolled in the Duke-UNC-Emory Acute HIV Consortium [11] and the GlaxoSmithKline-sponsored Quest study cohorts [12]. Included data from these cohorts were obtained at single time points before antiretroviral therapy. Semen data were not included for patients with evidence of concurrent sexually transmitted diseases (STDs). To develop a more precise model, viral dynamics in blood were assessed by combining blood data from these same cohorts with additional data on blood HIV-1 concentrations from individuals with well-characterized acute HIV infection that had been directly abstracted from the published literature [9]. When the date of infection for an individual patient was not known, the date of infection was estimated assuming a 14-day incubation period, on the basis of previously published data [13–16]. For all data on patients with acute infection, blood and semen HIV-1 concentrations were obtained and recorded before antiretroviral therapy, up to 1 year after HIV-1 infection. Informed consent was obtained from all participants; human experimentation guidelines of the US Department of Health and Human Services and/or those of all participating authors’ institutions were followed in the conduct of this research.

Patients with long-term HIV-1 infection. Developing the constructed model of viral dynamics in semen required us to estimate the distribution of semen HIV-1 concentrations for patients with chronic HIV-1 infection at virologic set point; for this purpose, data on semen HIV-1 load were collected from patients with chronic HIV-1 infection enrolled in previously published studies at the University of North Carolina at Chapel Hill and University Hospital in St. Gallen, Switzerland [1]. Data were included for patients who were HIV-1 antibody positive, had CD4+ cell counts > 200 cells/mm3, and had no documented concurrent STDs.

HIV-1 RNA measurements. HIV-1 RNA concentrations in semen plasma were determined by use of NucliSens HIV-1 QT (lower limit of detection, <400 copies/mL; NASBA; bioMerieux) [17], by use of a modification of the Roche Ultra-sensitive reverse-transcriptase polymerase chain reaction (lower limit of detection, <200 copies/mL), or by use of both assays. Roche PCR was performed as follows: 500 μL of seminal plasma was mixed with 500 μL of normal human plasma and was centrifuged for 90 min at 50,000 g. The upper 900 μL of the supernatant was discarded, and the pellet was resuspended in the remaining 100 μL and used in the Roche PCR Amplicor kit, according to the manufacturer’s descriptions. NASBA results were used for analysis when results of both assays were available; results for the 2 assays were similar for the subset of specimens on which both assays were run (P.L.V., unpublished data). HIV-1 RNA concentrations in blood plasma were determined by use of various commercially available assays.

Study Design

This retrospective cohort study approached the problem of the probability of transmission of acute HIV-1 infection as follows:

1. The relationship between HIV-1 concentrations and time was assessed in both semen and blood compartments, for patients donating both types of samples;
2. A precise model of viral dynamics in semen was constructed on the basis of longitudinally collected blood data and a hypothesized relationship between blood and semen compartments (i.e., parallel compartmental dynamics)—the prediction accuracy of the constructed model was then tested for agreement with observed data on semen HIV-1 concentration; and

3. The effect of predicted changes in viral dynamics in semen on the probability of transmission per coital act over time during acute HIV-1 infection was explored by combining the constructed model of viral dynamics in semen with a probabilistic model published elsewhere [1].

Statistical Methods

**HIV-1 RNA measurements.** Results for either compartment (blood or semen) that were undetectable were assigned a value that was one-half the absolute lower limit of detection for the assay that was used and for that compartment. All HIV-1 RNA data were then log-transformed before analysis.

**Descriptive viral dynamics in semen.** For patients with concurrent blood and semen HIV-1 load values, individual observations were plotted versus the time from infection. Correlation of blood and semen HIV-1 concentrations and trends in semen HIV-1 RNA concentration over time were assessed by use of Pearson's correlation. Descriptive regression models on the observed semen data were compared by use of the likelihood ratio test.

**Construction of model of viral dynamics in semen.** Because descriptive models were based on relatively few semen samples obtained from study subjects before the initiation of antiretroviral therapy, we constructed a model of viral dynamics in semen starting with more-abundant data on blood HIV-1 concentrations in samples obtained from untreated patients with acute HIV-1 infection: a piecewise polynomial linear mixed model [18] with a covariance structure based on random coefficients was first used to obtain the population average curve for blood data. Knots for the piecewise regression were chosen on the basis of a grid search for maximum likelihood estimators, with peak viremia estimated to occur on day 20, and the period of chronic viremia estimated to begin on day 54. The mean HIV-1 RNA load was assumed to be $-6.00 \log$ copies/mL on day 0, for consistency with previous reports [9]. A predictive average semen HIV-1 curve was made by adjusting the model of viral dynamics in blood so that the predicted set point matched the mean semen HIV-1 concentration for a population of 42 patients with chronic HIV infection and CD4 cell counts $>200$ cells/mm$^3$. The prediction accuracy of the final constructed model was assessed in terms of agreement between predicted and observed values, as measured by the number of observed data points falling within prediction bands around the predicted population curve in the final constructed model.

**Estimation of probability of transmission for hypothetical partnerships during acute HIV-1 infection.** To estimate the effect of changes in semen HIV-1 load on an individual's infectiousness during acute infection, probabilities of male-female transmission per coital act were calculated from predicted semen HIV-1 RNA load values, for hypothetical partnerships, by use of a probabilistic model published elsewhere [1]. As put forth by Chakraborty et al. [1], this model based estimates of probability of transmission per coital act on rates observed among HIV-serodiscordant couples and on an assumption that, for any individual partnership, this probability was primarily determined by the absolute R5 HIV-1 count per ejaculate, for the male partner, and by the CCR5$^+$ cervicovaginal receptor-cell density, for the susceptible female partner. By reconciling distributions for these parameters, which were observed in clinical studies, with observed transmission rates, Chakraborty et al. were able to estimate the effects of varying either parameter on the probability of transmission within hypothetical partnerships. To do the same, using the present data, we estimated inputs to the model of Chakraborty et al., assuming 100% R5 HIV-1 in semen during acute infection, 70% R5 in semen for patients with chronic infection [19], a median ejaculate volume of 2.30 mL [1], and a median cervicovaginal receptor-cell density of 184.8 CCR5$^+$ cells/mm$^3$ [20]. Predicted semen HIV-1 curves were generated by adjusting the curve from the constructed model on viral dynamics in semen to match (at day 54) R5 HIV-1 counts in semen for representative men at set point. The estimated probability of transmission for partnerships including these men were then plotted versus the time from infection. Probabilities of transmission within partnerships in which the partners had different frequencies of unprotected coitus during the period from day 0 to day 54 were calculated assuming regular coitus at evenly spaced intervals, beginning on day 0.

**RESULTS**

**Excretion of HIV-1 in semen during acute infection:** To assess the dynamics of the excretion of HIV-1 in semen during acute HIV-1 infection, we examined concurrent blood and semen HIV-1 RNA concentrations in samples from 30 men with acute HIV-1 infection participating in 2 large, acute-infection cohorts. All semen samples from subjects with acute infection were donated 14–84 days (median, 38 days) after the estimated date of infection. Semen HIV-1 RNA concentrations were similar between cohorts. Overall, in samples from men with acute infection, semen HIV-1 RNA concentrations (mean ± SD, 4.1 ± 1.14 log copies/mL) were significantly higher than those in samples from a comparison group of 42 antiretroviral treatment-naive men with chronic HIV-1 infection (mean ± SD, 3.49 ± 1.28 log copies/mL) ($P = .04$). Semen HIV-1 RNA con-
Figure 1. Regression model on semen data. Concurrent, pre-antiretroviral-therapy HIV-1 concentrations in semen (black circles) and blood (white circles) are shown plotted vs. the estimated time from infection, for men with acute HIV-1 infection without sexually transmitted diseases (n = 30). A cubic regression model on observed semen HIV-1 concentration data (solid black line) is shown, along with its 95% confidence intervals (interrupted black line).

Concentrations determined close to the time of the onset of symptoms were significantly higher than those determined later during acute infection (P < 0.01). The fitted cubic regression curve for the semen data (figure 1) closely resembled the form of previously published models [6–9] describing viral dynamics in blood; this curve had a statistically significantly better fit than did the alternate quadratic regression curve (P < 0.0001). In addition, we found that acute-infection blood and semen HIV-1 concentrations were significantly correlated over time, despite a likely rapid flux in these measurements within individuals (Pearson’s correlation, 0.37; P = 0.04).

Modeling viral dynamics in semen. Although these cross-sectional data suggested similar dynamics in blood and semen, the resultant descriptive model had insufficient prediction accuracy to allow estimation of the dynamics of the probability of transmission. However, we had access to abundant, longitudinally obtained blood data, which we used to develop a more precise model of viral dynamics in semen. We reasoned that, if the relationship between blood and semen HIV-1 concentrations were relatively constant within each individual in a population during acute infection, then the scatter of semen HIV-1 concentrations plotted versus time, for a population, would parallel changes in blood plasma viremia in the same population. We therefore constructed a predictive model of viral dynamics in semen, from blood data, assuming the hypothesized relationship, and then assessed the prediction accuracy of this constructed model in terms of agreement between predicted and observed semen HIV-1 RNA load values, following a paradigm frequently used to study population pharmacokinetics. A blood curve representing mean log_{10} HIV-1 RNA concentration was constructed on 171 longitudinal blood HIV-1 concentration data points, for 53 patients (mean follow-up, 12.4 days; median follow-up, 1 day; range, 1–80 days), by use of piecewise polynomial regression (figure 2) [18]. This curve demonstrated an initial rapid increase in blood HIV-1 load, reaching an estimated average peak HIV-1 RNA load of 5.93 log copies/mL at day 20 after infection (6 days after onset of symptoms, for patients with an acute retroviral syndrome) and declining to 4.74 log copies/mL by day 54 (day 40 after onset of symptoms). Assuming parallel dynamics in blood and semen, we constructed a predictive average semen probabilities curve by adjusting the model of viral dynamics in blood so that the predicted set point matched the mean semen HIV-1 concentration for patients with chronic HIV-1 infection and CD4 cell counts >200 cells/mm³. Finally, we measured the pre-
Acute HIV and Sexual Transmission

Figure 3. Observed and predicted semen HIV-1 RNA distributions. Semen plasma HIV-1 RNA concentrations (black circles) are superimposed on a prediction band representing the constructed model on viral dynamics in semen. Prediction bands were defined by adjusting the average fitted blood curve so that predicted values at day 54 (virologic set point) matched a distribution of semen HIV-1 concentrations in men with long-standing HIV-1 infection ($n = 42$). The band shown here is defined by the predicted population mean ± 1 SD.

Dynamics of the probability of transmission. To better understand the effect of the changes in semen HIV-1 load predicted by our constructed model of viral dynamics in semen on an individual's infectiousness during acute infection, we used the previously published probabilistic model of Chakraborty et al. [1], treating male-female sexual transmission of HIV-1 as a function of R5 HIV-1 count in an ejaculate and CCR5+ receptor-cell density in cervicovaginal tissues. We explored the effect of varying semen HIV-1 concentrations on the probability of transmission in a hypothetical partnership. By assuming the accuracy of the constructed model of viral dynamics in semen, we generated predicted semen HIV-1 curves for men with representative semen HIV-1 concentrations at day 54. We then derived estimates of the dynamics of the probability of transmission, assuming average susceptibility in a hypothetical partner (figure 4). We estimated that, at the time of peak viremia predicted by our constructed model (day 20 after infection), the probabilities of transmission per coital act, for individuals at the 25th, 50th, and 75th percentiles of the observed distribution of semen values for men at set point, were 1/1099, 1/213, and 1 transmission event/53 coital acts, respectively (table 1). For calculations of the probabilities of transmission within hypothetical partnerships in which the partners had different frequencies of unprotected coitus during the period from day 0 to day 54, we assumed average susceptibility of the partner, no change in the susceptibility of the partner during this time, and regular coitus at evenly spaced intervals, beginning on day 0. The probabilities of transmission within stable partnerships, assuming 8 coital acts/month over the course of 54 days of infection, were 0.6%, 3.2%, and 12.4%, respectively, for the same categories of individuals as above.

DISCUSSION

The present study has provided empirical evidence that men with acute HIV-1 infection are biologically hyperinfectious because of increased genital shedding of HIV-1. In addition, the present study has provided evidence that, during acute infection, HIV-1 load increases and decreases in semen in approx-
acute infection should be considered as minimal estimates, for several additional reasons. First, average fitted curves for populations tend to blunt individual peak values when individual curves are asynchronous—which is almost certainly the case in patients in our study and, hence, in our final constructed model. Second, overall, genital fluid HIV-1 inoculum is only 1 potentially important biological determinant of individual infectiousness. A given inoculum of HIV-1 may be expected to have higher infectious potential during acute infection, since early variants (typically R5) are very homogeneous in env and are closely related to successfully transmitted strains. The absence of antibodies to HIV-1 in infectious body fluids early during acute infection might also increase the potential of transmission. Third, other factors related to increased susceptibility of the partner (e.g., frequent genital ulceration or absence of acquired mucosal immunity [22–24]) or high rates of partner change and riskier modes of sexual interaction [25] would be expected to amplify the effect of increased infectiousness during acute HIV-1 infection on probability transmission and spread within sexual networks [26].

A number of lines of epidemiologic evidence suggest that acute infection fuels heterosexual spread of HIV-1. Both look-back studies examining transmission rates [27, 28] and case series documenting rapid secondary transmission [13, 29] have suggested an elevated risk of transmission per coital act, relative to chronic infection. In addition, mathematical modeling studies [26] and evidence of extensive case-clustering among acutely infected patients [29] have demonstrated that acute HIV-1 infection may play an important role in the pandemic.

The present study used cross-sectional data and cannot account for possible selection bias with regard to the time samples were obtained from patients. It is also not possible to completely evaluate the influence of potential confounders, such as STDs or differences in viral subtype or phenotype, in the study pop-

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<tr>
<th>Semen HIV-1 RNA concentration percentile, days from infection</th>
<th>Semen HIV-1 RNA concentration, log_{10} HIV-1 RNA copies/mL</th>
<th>Point estimate of probability of HIV-1 transmission/coital act (95% confidence bound)</th>
<th>Estimated odds of HIV-1 transmission/coital act</th>
<th>Fold change (day 20–54)</th>
<th>Probability of transmission during acute phase (day 0–54) in a partnership with a given frequency of unprotected coitus</th>
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ulation. In addition, patients with symptomatic, acute HIV-1 infection may have higher than average blood HIV-1 loads; however, in the present study, higher blood HIV-1 load values would not necessarily lead to overestimation of the probability of transmission, given the way that our particular model was constructed. Moreover, the present study’s weaknesses are counterbalanced by important strengths. By pooling longitudinally collected blood data from multiple studies, we were able to develop a model of viral dynamics for a population, with precision and predictive power. The hypothesis-driven statistical approach that we used to assess viral dynamics in semen in the present study may have promise in assessing other clinical situations and difficult-to-sample tissue compartments, such as the female genital tract or the central nervous system. Finally, the present study directly relates measured changes in genital HIV-1 shedding to probability of transmission, by use of the empirically derived model of Chakraborty et al. [1]. The projections derived from the model of Chakraborty et al., on the basis of our estimates of viral dynamics in semen, cannot account for the influence of variations in the susceptibility of female partners; nonetheless, the ability to estimate the effect of measurable biological phenomena on the probability of HIV-1 transmission is a powerful tool that could be useful in modeling HIV-1 prevention strategies. In particular, the observation that, in the absence of STDs, viral dynamics in blood during acute HIV infection may accurately reflect viral dynamics in the genital tract allows more-confident exploration of the effects of interventions early during HIV disease on transmission in populations.

Only a small number of the estimated 40,000 acute HIV-1 infections that occur annually in the United States [30] are diagnosed, and there are few specific public health systems in place to facilitate identification or management of the syndrome. It has been assumed that, because of the brevity of acute infection, individuals with acute infection would make a minor contribution to the epidemic. However, the efficiency of transmission predicted by the present study forces reconsideration of such assumptions and underscores acute HIV-1 infection as a unique public health opportunity [31]. This may be particularly true in sub-Saharan Africa. Public health interventions directed at acutely infected individuals, including safe-sex counseling, condom promotion, and antiretroviral therapy, can decrease sexual infectivity to the extent that sexual practices are altered and/or HIV-1 load is reduced over time. These effects are maximized when introduced early during infection and at a time of high transmission potential. The potential clinical benefits [32] of acute antiretroviral treatment for individual patients who are diagnosed during antibody-negative acute infection further emphasize the need for improved and early identification of cases of HIV-1 infection. Treatment considerations for recent sex contacts should include both prospective screening for acute and chronic HIV infection and appropriate screening for other sexually transmitted pathogens. An estimated average probability of sexual transmission of 1 transmission event/213 coital acts—a risk of HIV-1 acquisition similar to that associated with percutaneous blood exposures in the setting of chronic HIV-1 infection [33]—may further warrant rapid identification and notification of potentially exposed partners, with possible provision of post-sexual-exposure prophylaxis.

The benefits potentially associated with diagnosis of acute HIV-1 infection are likely to be greatest if cases of infection are identified around the period of peak viremia, a circumstance that is, at present, rare. Public health strategies that may aid in the identification of early acute infections include incorporating pooling and nucleic acid screening into routine HIV-1 testing [34] and developing more-effective acute HIV-infection referral networks [12]. These efforts may make it possible to implement a proactive response to transmission by patients with acute HIV-1 infection and to facilitate these patients’ early entry into care.

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**QUEST CORE GROUP AND RESEARCH TEAM**

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