The Relation Between Symptoms, Viral Load, and Viral Load Set Point in Primary HIV Infection

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Objectives: To examine the relation between symptoms, initial viral load, and viral load set point in primary HIV infection (PHI).

Design: Prospective cohort of patients with preseroconversion or recent seroconversion HIV infection (typically <60 days) in San Francisco.

Methods: Subjects were questioned about 21 potential PHI symptoms at enrollment and were subsequently followed with viral load measures.

Results: The analysis included 57 subjects with preseroconversion HIV infection and 120 with recent seroconversion. In univariate analysis, most symptoms and the total number of symptoms were each associated with a significantly higher initial viral load. In stepwise multiple linear regression, however, only the number of symptoms was independently associated with a higher initial viral load, with an increase in the initial viral load of 0.08 $\log_{10}$ per additional symptom ($P < 0.001$). In univariate analysis, more PHI symptoms were associated with a higher viral load set point, but in a multivariable mixed-effects model, this association was accounted for by the initial viral load, which was strongly correlated with viral load set point ($R = 0.44$, $P < 0.001$).

Conclusions: A high initial viral load was associated with more symptoms during PHI. The strong correlation between initial HIV-1 RNA viral load levels and viral load set point suggests that early interactions between the HIV-1 virus and a new host, even before fully developed adaptive immune responses, are important in establishing viral load set point.

Key Words: HIV, HIV natural history, primary HIV infection, primary HIV symptoms, viral load set point

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Primary HIV infection (PHI) can include a self-limited period of flu-like symptoms occurring during the first weeks of HIV-1 infection.[1] It is associated with peak levels of HIV-1 RNA viremia, which subsequently decline until reaching a set point, where levels remain for months to years.[2,3] Initial studies suggested that those with more symptomatic acute infection and longer duration of illness have faster rates of progression to AIDS.[4–7] The presence of symptoms has conversely been correlated with faster clearance of the virus, however, suggesting that symptoms may indicate a beneficial immunologic response.[8] In addition, data from a cohort of acutely infected African women have associated more symptomatic disease with higher preseroconversion viral loads and increases in mortality independent of viral load set point.[9,10] Nonetheless, previous studies have not included data on initial viral load, PHI symptoms, and viral load set point together, and the interrelations between these 3 factors remain unclear. We sought to address the relation between viral load and PHI symptoms and the association of both of these factors with viral load set point in a cohort enrolled before or within approximately 2 months after HIV antibody seroconversion.

METHODS

The University of California at San Francisco (UCSF) Options Project recruited subjects with possible PHI who met 1 of 2 criteria: potential preseroconversion or recent HIV antibody seroconversion. Detailed referral methods have been reported elsewhere.[11] The Options Project protocol was approved by the UCSF Institutional Review Board, and informed consent was obtained from all participants.

Participants were classified as preseroconversion HIV infected if (1) results of testing using standard enzyme immunoassay (EIA) HIV-1 antibody testing and confirmatory Western blot analysis were negative or indeterminate and (2) an HIV-1 RNA test showed >2000 copies/μL on 2 or more tests. Participants were classified as recent seroconversion recently infected if they were positive for HIV-1 antibody with a history compatible with recent HIV infection (all prior HIV antibody test results were negative by subject report, and there were recent potential HIV exposures) and a less-sensitive EIA (LS-EIA) test result[12] with an optical density (OD) <0.2. The LS-EIA uses a modified EIA test that typically takes 6 months after standard antibody test seroconversion to reach an OD of 1.0;[12,13] the lower cutoff of 0.2 OD selects subjects who are typically within 4 weeks of seroconversion.[14]

Participants were asked whether they had recently experienced any of 21 symptoms: fever, rash, oral ulcers (mouth

sores), arthralgias (joint pain), pharyngitis (sore throat), loss of appetite, lost weight (>5 lb [2.5 kg]), malaise (felt sick), myalgias (pain in muscles), tired or fatigued, nausea, headaches, photophobia, night sweats, confusion, infected gums, diarrhea, sores on genitals, vomiting, sores on anus, and stiff neck. For each symptom experienced, participants were asked start dates and duration of symptoms. Symptoms that occurred more than 60 days before or after the estimated time of infection were excluded from the analysis, as were symptoms that occurred for longer than 45 days. This step was taken to exclude symptoms that were likely to be chronic or unrelated to PHI, whereas allowing for error in the estimated time of infection.

Plasma HIV-1 RNA was measured using a branched DNA (bDNA) 2.0 assay until January 1999 and a 3.0 assay thereafter (Bayer HealthCare Diagnostics, Berkeley, CA). After study entry, HIV-1 RNA was measured every 4 weeks during the first 24 weeks of the study and every 8 weeks thereafter during the period of observation used in this report. The date of HIV infection was estimated based on prior data on the median time from exposure to PHI symptoms; an indeterminate HIV-1 antibody test result[15] at the midpoint between the last negative and first positive HIV-1 antibody test results; or the level of a less sensitive HIV-1 antibody test if the OD was between 0.5 and 1.0, a range in which there has been shown to be a linear relation between the LS-EIA antibody results and the days since seroconversion.[13]

Statistical analysis was performed using SAS version 9 (SAS Institute, Cary, NC). Viral load, number of days symptomatic, and time from infection to initial viral load measurement were log-transformed to achieve a more normal distribution. Viral loads reported at <500 copies/mL were set to 250 copies/mL to allow for variation in detection level for the 2 versions of viral load tests used. For analysis of differences between means, *t* tests were used. To test the association of initial screening viral load with symptoms, we used univariate and multivariate linear regression modeling. Finally, for subjects who were not treated in early HIV infection, mixed-effects modeling was used to predict viral load set point, defined as viral load measures between weeks 24 and 108 of follow-up. We confirmed this period as a good estimate of viral load set point by including a variable in the models representing the time in weeks from study entry to follow-up for each viral load measurement. The mixed-effects model we used, employing Proc Mixed in SAS, estimated average viral load over this period, while accounting for variation in the length of observation and differences in frequency of measures attributable to missed visits (random effect for time) and individual variation in viral load at week 24 (random effect for intercept).

RESULTS

Fifty-seven subjects defined as having preseroconversion HIV infection and 120 subjects classified as having recent seroconversion infection were included in the analysis. Almost all were homosexual men, representing the epidemiology of HIV infection in San Francisco, and the average age of the subjects was 41 years. (Supplemental materials are available via the Article Plus feature at www.jaids.com. You may locate this article, then click on the Article Plus link on the right; for details of subject characteristics in both groups.) Subjects with preseroconversion HIV presented with significantly more symptoms compared with those with recent seroconversion (mean: 9 vs. 6; $P < 0.001$) and a higher $\log_{10}$ viral load than those with recent seroconversion (mean: 5.1 vs. 4.4; $P < 0.001$). Approximately 20% of those with recent seroconversion were asymptomatic, compared with only 3.5% of those with preseroconversion, representing differences in recruitment for the 2 groups. Both groups were symptomatic for a mean of 10 days. Fatigue, malaise, headache, fever, night sweats, pharyngitis, anorexia, rash, arthralgias, and myalgias were all present in >50% of acutely infected individuals (Fig. 1). Although the frequency of these symptoms was lower in persons diagnosed with recent seroconversion, with the exception of rash, these symptoms were also present in >30% of those with recent seroconversion.

An increase in the number of symptoms was associated with a higher initial viral load in the preseroconversion and recent seroconversion groups (Fig. 2). In univariate regression models, each additional symptom predicted a 0.09 $\log_{10}$ higher viral load ($P < 0.001$). The presence of symptomatic versus asymptomatic infection was associated with a 0.9 $\log_{10}$ higher initial viral load ($P < 0.001$). The duration of symptoms was not associated with a higher initial viral load.

All individual symptoms assessed, with the exception of anal and genital sores, infected gums, and photophobia, were associated with a statistically significant higher initial viral load in univariate analysis. To assess which symptoms were most important in predicting initial viral load, all symptoms with a *P* value <0.1 in univariate analysis and the stage of infection (preseroconversion vs. recent seroconversion) were assessed in a stepwise linear regression model. Preseroconversion infection was associated with a 0.5 $\log_{10}$ higher viral load ($P = 0.001$). The presence of fever was associated with a 0.6 $\log_{10}$ higher viral load ($P < 0.001$), and anorexia was

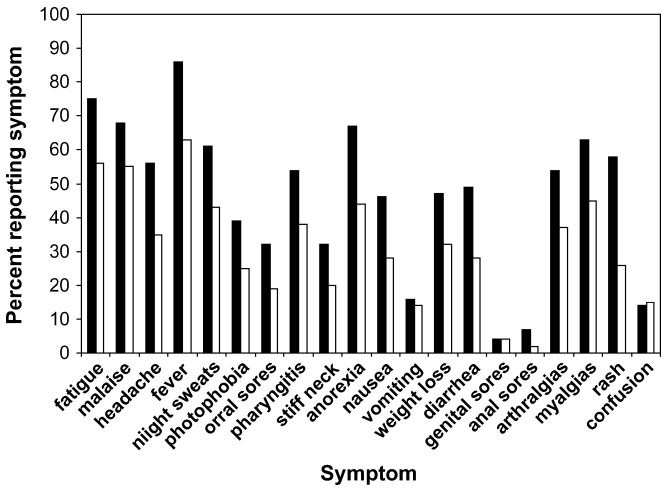

FIGURE 1. Frequency of self-reported acute HIV symptoms. Solid bars represent preseroconversion subjects (n = 57), and hollow bars represent postseroconversion subjects (n = 120).

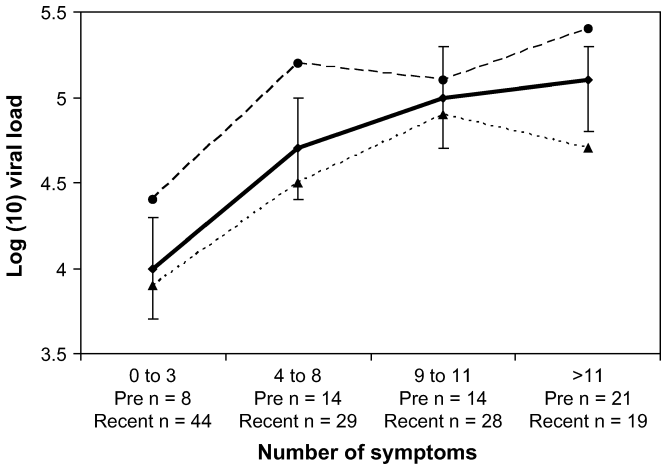

FIGURE 2. Mean initial viral load based on number of symptoms (categorized in quartiles) and stratified by whether subjects had preseroconversion HIV or recent seroconversion. Subjects with preseroconversion infection are represented by a dashed line with circles, subjects with recent seroconversion infection are represented by a dotted line with triangles, and the combined groups are represented by a bold line with vertical bars for the intraquartile ranges. The regression coefficient for the preseroconversion group is 0.24 (P = 0.01), the regression coefficient for the recent seroconversion group is 0.35 ($P < 0.001$), and the regression coefficient for the combined groups is 0.38 ($P < 0.001$). Pre indicates preseroconversion; recent, recent seroconversion.

associated with a 0.4 $\log_{10}$ higher viral load ($P = 0.008$); other symptoms were not statistically significant in the stepwise model. Finally, all symptoms with P values <0.1, the stage of infection, the number of symptoms, and the duration of symptoms were assessed in a stepwise linear regression model with the initial viral load as the outcome. The only significant predictors of initial viral load in this model were preseroconversion infection, which was associated with a 0.48 $\log_{10}$ higher viral load ($P = 0.002$) compared with recent seroconversion, and the number of symptoms, for which each additional symptom was associated with a 0.08 $\log_{10}$ higher viral load ($P < 0.001$).

We performed univariate and multivariate mixed-effects modeling to predict viral load set point in the 48 subjects, 6 with preseroconversion and 42 with recent seroconversion, who did not start early antiretroviral treatment (Table 1). The median number of follow-up viral load measurements used to determine set point in the model was 5, and the median number of weeks of follow-up was 98. In univariate analysis, a 1.0 $\log_{10}$-increase in initial viral load predicted a 0.46 $\log_{10}$ higher viral load set point ($P < 0.001$), and each additional PHI symptom reported predicted an increase of 0.05 $\log_{10}$ viral load ($P = 0.03$). The duration of symptoms was not associated with viral load set point. In univariate modeling, each additional week from study entry to the follow-up measurement was associated with a small (0.003 $\log_{10}$ copy/mL per week) but significant ($P = 0.02$) increase in viral load set point, indicating that viral load slowly increased, on average, during this follow-up period. In multivariate modeling controlling for

TABLE 1. Results of Univariate and Multivariate Mixed Model to Predict Set Point Viral Load

	Univariate		Multivariate	
Variable	Regression Coefficient	P	Regression Coefficient	P
Screening viral load	0.46	<0.001	0.44	0.003
No. symptoms	0.05	0.04	−0.02	0.46
Recent seroconversion	−0.42	0.19	−0.07	0.80
Mean duration of symptoms in days	0.48	0.21	0.44	0.19
Time from study entry to follow-up measurement in weeks	0.003	0.02	0.002	0.85

preseroconversion versus recent seroconversion and time from study entry to viral load measure, however, a higher initial viral load remained significantly associated with a higher viral load set point and was the only statistically significant predictor (0.44 $\log_{10}$-copy/mL increase in viral load set point per 1.0 $\log_{10}$-copy/mL greater initial viral load; $P = 0.003$).

DISCUSSION

The number of PHI symptoms experienced was strongly associated with initial viral load in persons first evaluated before seroconversion or recently after seroconversion. This confirms prior reports suggesting that more symptomatic PHI is associated with a higher initial viral load.[10] The number of symptoms experienced was a stronger predictor of initial viral load than which individual symptoms were present. The effect of each additional symptom on viral load was more modest in our study than in a prior study by Lavreys et al (0.4 vs. 0.08 $\log_{10}$).[10] This difference may be explained, in part, by the extremely low viral load (364 copies/mL) observed in asymptomatic subjects in this earlier study, some of whom may have been captured during monthly follow-up visits before the "ramp-up" period of viremia.

Prior studies of PHI and progression to AIDS have suggested that the number of symptoms, duration of symptoms, and severity of symptoms all predict faster progression to AIDS.[4–7] Earlier studies have not been able to assess the relations between the presentation of symptoms during PHI, early viral load, and viral load set point. In our study, the number of PHI symptoms was associated with a higher viral load set point, a well-established marker of more rapid disease progression.[16,17] In a multivariable model, however, the association of PHI severity with viral load set point was accounted for by the association of a higher initial viral load with more PHI symptoms; initial viral load was the only independent predictor of viral load set point. For clinicians, our results suggest that a low viral load during PHI or soon after seroconversion is a more reliable indicator of which individuals are likely to achieve a low viral load set point than the severity of PHI.

A limitation of our study is that our population was mostly homosexual men; therefore, the results may not be generalizable to women. Detecting statistically significant

differences in associations between the initial viral load and individual symptoms was difficult, because many symptoms seem to be colinear (eg, malaise, anorexia). Despite controlling for stage of infection, there were only 6 subjects included in the set point analysis who were preseroconverters, suggesting that these results should be replicated in future studies in this subgroup.

Our results suggest that early interactions between the HIV-1 virus and a new host, even before fully developed adaptive immune responses, are important in establishing viral load set point. To focus on early interactions, we limited seroconverters to those with an LS-EIA OD <0.2, most of whom were likely to have been within 4 weeks of seroconversion. Early adaptive immune responses may have influenced the viral load at the first measurement made in our cohort, especially in those diagnosed during early HIV seroconversion. Although it is difficult to discern the degree to which early adaptive immune responses account for the association of initial viral load measures in our cohort with subsequent viral load set point, we believe our findings suggest that innate immune factors and/or HIV viral characteristics may have contributed to determining viral load set point and subsequent disease course. This is supported by finding similar trends in the association of initial viral load with subsequent viral load set point when restricting our analysis to participants who were first evaluated before seroconversion, although the small number of persons in this group precludes any clear conclusions and it is possible that early adaptive immune responses were already modifying viral load before antibody seroconversion. Our results are also consistent with those of 8 patients reported by Lindback and colleagues,[3] in whom there was an association between peak viral load in acute HIV and subsequent viral load levels more than 50 days later.

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