Association Between HIV-1 RNA Level and CD4 Cell Count Among Untreated HIV-Infected Individuals

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Objectives. We examined the significance of plasma HIV-1 RNA levels (or viral load alone) in predicting CD4 cell decline in untreated HIV-infected individuals.

Methods. Data were obtained from the British Columbia Centre for Excellence in HIV/AIDS. Participants included all residents who ever had a viral load determination in the province and who had never taken antiretroviral drugs (N=890). We analyzed a total of 2074 viral load measurements and 2332 CD4 cell counts. Linear mixed-effects models were used to predict CD4 cell decline over time.

Results. Longitudinal viral load was strongly associated with CD4 cell decline over time; an average of 1 $\log_{10}$ increase in viral load was associated with a 55-cell/mm^3 decrease in CD4 cell count.

Conclusions. Our results support the combined use of CD4 cell count and viral load as prognostic markers in HIV-infected individuals before the introduction of antiretroviral therapy. (*Am J Public Health.* 2009;99:S193–S196. doi:10.2105/AJPH.2008.137901)

The natural history of HIV disease is highly influenced by complex interactions between the virus and the host.[1–6] Interpatient variability is apparent in measurements of the time between seroconversion and the diagnosis of AIDS among untreated individuals. The average latency period between seroconversion and the diagnosis of AIDS is 10 years (±2 years), but some individuals progress to AIDS within 1 year, and others have not developed AIDS after 20 years of HIV infection.[1] This variability has been shown to be a function of genotypic differences in the virus[1,7–9] and in its hosts, in particular the capacity of individual immune systems to tolerate and control HIV replication.[1–6] From a clinical standpoint, it is important to understand an individual's short-term risk for disease progression, because this has profound implications for therapeutic management decisions.

Following the work of Mellors et al.,[10] clinicians usually calculate the risk of disease progression from a combination of CD4 cell count and HIV-1 RNA plasma viral load (or viral load alone). The CD4 cell count is generally regarded as a marker of the degree of immune deficiency, and the viral load is generally considered to be a marker of disease activity, or the rate at which the CD4 cell count is likely to decrease. The study by Mellors et al.[10] was also important because it showed that viral load provides prognostic information about the risk of developing conditions that are part of the definition of active AIDS, independently of CD4 cell count. That study demonstrated that individuals with a higher viral load during the asymptomatic phase in the absence of antiretroviral therapy (ART) were more likely to rapidly progress to AIDS. The findings were consistent with an Amsterdam cohort study of HIV serum conversion by Wolf et al.[11] and with an analysis by Lefrère et al. of data from a large cohort of HIV-infected persons in France.[12]

International guidelines have consistently incorporated these 2 key laboratory tests for monitoring HIV-infected individuals and determining the timing of ART.[13–21] A recently published study, however, challenged this practice. Rodriguez et al. analyzed data from HIV-infected individuals enrolled in the Research in Access to Care for the Homeless Cohort, the San Francisco Men's Health Study, and the Multicenter AIDS Cohort Study.[22] A total of 1289 patients were enrolled between 1984 and 2004 and followed for an average of 2.3 years. In brief, the authors found that presenting viral load explained less than 10% of the variability in the slope of CD4 cell decline before therapy initiation; they concluded that viral load was an imprecise predictor of CD4 cell decline and that viral load could not explain the between-individual variation in the rate of CD4 cell decline. The controversial conclusions of this study kindled a lively debate among researchers. Some investigators advised that the findings by Rodriguez et al. should be interpreted with caution because of either methodological shortcomings or failure to separately investigate the role of viral load as a prognostic factor in CD4 cell depletion and in the emergence of conditions among untreated patients that are part of the definition of active AIDS.

We sought to shed further light on this controversy by characterizing the extent to which viral load predicts CD4 cell decline among HIV-infected individuals before initiation of treatment. We felt that it would be premature to conclude that baseline viral load is a poor predictor of the rate of CD4 cell decline among untreated HIV-infected individuals and that other independent natural history studies would be of vital importance. We used longitudinal statistical methods to construct a model of CD4 cell count depletion.

METHODS

HIV Patients in British Columbia

Since October 1992, the distribution of antiretrovirals to HIV-infected persons in British Columbia has been the responsibility of the HIV/AIDS Drug Treatment Program of the British Columbia Centre for Excellence in HIV/AIDS. ART is provided at no cost to all eligible HIV-infected individuals according to specific guidelines generated by the Therapeutic Guidelines Committee. Since June 1996, the center adopted viral load–driven ART guidelines consistent with those proposed by the International AIDS Society–USA.[13–18]

The AIDS case definition in British Columbia is based not on CD4 cell count or HIV-1 RNA plasma viral load but rather on the extensive Centers for Disease Control and Prevention list of AIDS conditions.[18,23–25] Thresholds for therapy initiation are based on either CD4 cell count or CD4 cell fraction.[18,23] If an individual has

a clinical AIDS diagnosis, a CD4 cell count of 200 cells/mm^3 or less or a CD4 fraction of 15% or less, treatment is initiated. If the CD4 cell count is between 200 and 350 cells/mm^3 or the CD4 fraction is 15% or less, treatment is advised. Finally, if the CD4 cell count is more than 350 cells/mm^3 or if the HIV disease is in its primary HIV infection stage, treatment is deferred.

We extracted data from the Antecedent HIV-1 Plasma Viral Load Monitoring Program, in which clients were enrolled by physician request and had their viral load and CD4 cell count regularly but nonsystematically monitored. This program included individuals before their enrollment in the center's HIV/AIDS Drug Treatment Program. Medical and laboratory monitoring (viral load and CD4 cell count) is free of charge in the province, and the center's treatment guidelines recommend that clients be tested at least quarterly.

Our analyses were restricted to individuals who had their first viral load test after 1996 and who started therapy during 2000 to 2004 (N=890). Participants were eligible if they were 18 years or older and had never received ART; participants were censored when they met eligibility criteria for treatment. Viral load measurements were obtained with the standard Roche viral load assay (range=500–10^6 copies/mL) until 1999 and with the ultrasensitive Roche assay (range=50–10^5 copies/mL) thereafter. Therefore, viral load measurements were recoded to range from 500 to 10^5 copies/mL, to standardize the viral load range over time. CD4 cell counts were measured by flow cytometry (Beckman Coulter, Inc, Mississauga, Ontario).

CD4 Depletion Analysis

Matched CD4 cell counts and viral loads were obtained from the center's database. All viral load tests performed in British Columbia are assayed at the St Paul's Hospital Virology Laboratory. Approximately 90% of all CD4 cell counts in British Columbia are performed at the Flow Cytometry Laboratory at St Paul's Hospital, with the remaining 10% performed at other laboratories in British Columbia.

We extracted CD4 cell count and viral load data from patient records for 6-month intervals from the first measurement until therapy initiation date, last contact date, or date of death. We searched for the test results that were recorded closest to the beginning of each 6-month interval. The statistical analyses adjusted for age at the start of follow-up, gender, and viral load ($\log_{10}$ transformed). These were the only covariates available in the center's database.

The statistical analyses estimated the change in CD4 cell count over time via linear mixed-effects models adjusted for potential explanatory variables, including viral load, baseline CD4 cell count, time, age, and gender.[26–28] Linear mixed-effects models were used because they take into account the inter- and intrapatient sources of variation, they are flexible enough to account for the natural heterogeneity in the population, and they can handle any degree of imbalance in the longitudinal data.[26–28] The resulting estimated b, the fixed-effect parameter for each predictor in these models, represents the average change in CD4 cell count for a unit increase in that predictor. In the first stage of a linear mixed-effects model, the general structure for the mean response model $[E(.)]$ is given by

$$E(CD4_{it}) = \alpha_{0,i} + \beta_1 \times \text{gender}_i + \beta_2 \times \text{age}_i + \beta_3 \times \text{baseline CD4 cell count}_i + \beta_4 \times \text{time}_{it} + \beta_5 \times \log_{10}(\text{viralload}_{it}),$$

where $\alpha_{0,i}$ is a random-effect intercept that varies according to i, which is the patient index; t is the time and has the value 0 (start of follow-up) to 1 (12 months), 2 (24 months), 3 (30 months), or 4 (therapy initiation date, last contact date, or date of death); and $b_1, \ldots, b_5$ are fixed-effect parameters associated with the nonrandom predictors. In this model, age, baseline CD4 cell count, and viral load ($\log_{10}$ transformed) were considered continuous variables. Viral load ($\log_{10}$ transformed) and time (i.e., time when CD4 cell count was performed) were considered time-updated variables. Time was included in the model to allow for nonlinearity. In the second stage of this model, the relationship between different CD4 cell counts over time was defined. Because 2 adjacent CD4 cell counts are highly correlated and this correlation diminishes as the interval between measurements increases, it is reasonable to assume a first-order autoregressive covariance pattern.[26–28]

A sensitivity analysis was also conducted to eliminate the possibility of some individuals being enrolled in our study during their primary or acute infection period, when very high viremia could skew the data. We therefore excluded participants (1.28%) who experienced a greater than 1 $\log_{10}$ decrease in viral load between the start of follow-up and the first of the 6-month intervals.

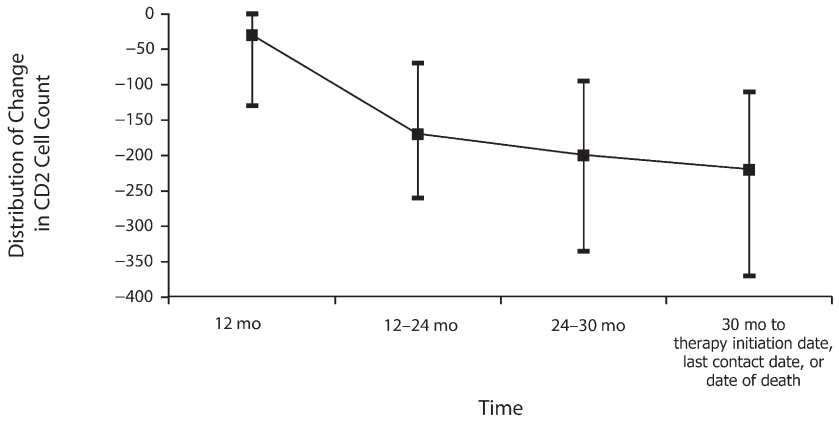
RESULTS

A total of 2074 viral load measurements and 2332 CD4 cell counts were included in the analyses. The median age of the patients was 39 years (interquartile range=34–46 years), 81% were male, the median CD4 cell count was 410 cells/mm^3 (interquartile range=290–560 cells/mm^3), and the median viral load was 4.7 $\log_{10}$ copies/mL (interquartile range=4.1–5.0 $\log_{10}$ copies/mL). The median follow-up was 16.9 months (interquartile range=9.7–34.0 months; range=3.4–97.3 months). Figure 1 shows the distribution of change in CD4 cell count over time. CD4 cell loss accelerated over time, with a median decrease of 30 (interquartile range=−130 to 0 cells) in the first year of follow-up and a median decrease of 220 (interquartile range=−370 to −110 cells) in the last year of follow-up.

Table 1 summarizes the parameter estimates of CD4 cell count trajectory according to the mixed-effects model for each of the follow-up periods (start of follow-up until 12 months, 24 months, and 30 months or until therapy initiation date, last contact date, or death). The model shows that viral load was strongly associated with CD4 cell decline at all times. For example, the model for change between baseline and therapy initiation date, the last contact date, or date of death indicates that on average for every 1 $\log_{10}$ increase in viral load there was an average additional loss of 55 CD4 cells (b=−54.58; SE=6.36; P<.01]). In addition, we estimated that from baseline to therapy initiation date, last contact date, or date of death, the percentage change in CD4 cell count was −75%.

DISCUSSION

Our results support the continued use of viral load for estimating prognosis in untreated HIV individuals, as first proposed by Mellors et al. in 1997.[10] We demonstrated that measurements of viral load were strong predictors of CD4 cell decline over time, and this association became stronger with continued follow-up. These results also confirm the strong predictive value of a baseline CD4 cell count.



Note. Measurements were taken at the start of follow-up; at 12 months, 24 months, and 30 months; and at the therapy initiation date. The vertical bars indicate the interquartile range.

FIGURE 1—Distribution of change in CD4 cell count over time among untreated HIV-infected individuals: British Columbia, 2000–2004.

Our results conflict with the recently published report by Rodriguez et al.[22] Several factors might account for the discordance between our studies. Intraindividual variability in CD4 cell counts and viral load can be quite substantial,[29] and the signal-to-noise ratio associated with these measurements may have obscured correlations in the short follow-up time examined by Rodriguez et al.[22] Our analysis incorporated a longer follow-up time, and all viral load tests were conducted at a single laboratory. We also were able to reliably exclude any results obtained after the initiation of ART, because our program is the only source of antiretroviral drugs in British Columbia.

Several features of our study are significant. First, we had a long follow-up, and the mixed-effects model was able to control for intra- and interindividual variations, in addition to handling highly imbalanced data. Second, CD4 cell count and viral load are vulnerable to measurement biases.[30,31] Our viral load measurements were obtained from the St Paul's Hospital Virology Laboratory, which performs all such tests in the province. CD4 cell counts from approximately 90% of all available test results in our study were obtained from the St Paul's Hospital Flow Cytometry Laboratory. Thus, we believe that measurement bias was minimal in our data. Third, the British Columbia Centre for Excellence in HIV/AIDS is responsible for the only population-based program for testing viral load in British Columbia; therefore, our results may be broadly applicable to other populations. Fourth, this study was restricted to individuals who had not received ART, thus eliminating any possible confounding effect of previous ART exposure.

Although we adjusted our analyses for important covariates, they may have been subject to residual confounding because of unmeasured covariates, or because our data came from an observational study, or because other factors influenced the decision to start treatment. Caution in interpreting these findings is therefore warranted.

We found viral load to be a significant predictor of the rate of CD4 cell decline among untreated HIV-infected individuals. Moreover, the predictive value of viral load increased with longitudinal follow-up in our cohort of HIV-infected individuals who had never received ART. Our results support the combined use of CD4 cell count and plasma viral load as prognostic markers in HIV-infected individuals who have not yet begun ART. ■

TABLE 1—Parameter Estimates for CD4 Cell Count Trajectory in Mixed-Effects Model

	Intercept, b (SE)	CD4 Cell Count at Start of Follow-up, b (SE)	HIV-1 RNA Plasma Viral Load (log_{10} transformed), b (SE)	Time, b (SE)
Model 1[a]	814.36 (51.39)	. . .	−85.93 (11.41)	. . .
Model 2[b]	351.09 (36.56)	0.65 (0.02)	−48.87 (7.25)	−39.60 (5.90)
Model 3[c]	384.47 (34.66)	0.62 (0.02)	−52.41 (6.87)	−45.52 (4.39)
Model 4[d]	404.11 (32.88)	0.60 (0.02)	−55.90 (6.60)	−41.00 (3.36)
Model 5[e]	428.21 (31.59)	0.57 (0.02)	−54.58 (6.36)	−51.51 (2.55)

Note. Data are from the Antecedent HIV-1 Plasma Viral Load Monitoring Program of the British Columbia Centre for Excellence in HIV/AIDS (N = 890). We fit 5 different models for each 6-month interval of follow-up. Depending on the model being fitted, the time variable had the value 0 (start of follow-up) to 1 (12 months), 2 (24 months), 3 (30 months), or 4 (therapy initiation date, last contact date, or date of death).
[a]Included CD4 count, viral load, and time measurements at the start of follow-up.
[b]Included baseline CD4 count, time, and viral load at the start of follow-up and at 12 months.
[c]Included baseline CD4 count, time, and viral load at the start of follow-up and at 12 and 24 months.
[d]Included baseline CD4 count, time, and viral load at the start of follow-up and at 12, 24, and 30 months.
[e]Included baseline CD4 count, time, and viral load at the start of follow-up; 2, 24, and 30 months; and therapy initiation date, last contact date, or date of death.

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Contributors

All authors contributed to the conception and design of this study, the interpretation of the data, and the editing

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Human Participant Protection

The Centre's HIV/AIDS Drug Treatment program has received ethical approval from the University of British Columbia ethics review committee at its St Paul's Hospital site.

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