Cumulative Viral Load and Virologic Decay Patterns after Antiretroviral Therapy in HIV-Infected Subjects Influence CD4 Recovery and AIDS

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Abstract

**Background:** The impact of viral load (VL) decay and cumulative VL on CD4 recovery and AIDS after highly-active antiretroviral therapy (HAART) is unknown.

**Methods and Findings:** Three virologic kinetic parameters (first year and overall exponential VL decay constants, and first year VL slope) and cumulative VL during HAART were estimated for 2,278 patients who initiated HAART in the U.S. Military HIV Natural History Study. CD4 and VL trajectories were computed using linear and nonlinear Generalized Estimating Equations models. Multivariate Poisson and linear regression models were used to determine associations of VL parameters with CD4 recovery, adjusted for factors known to correlate with immune recovery. Cumulative VL higher than the sample median was independently associated with an increased risk of AIDS (relative risk 2.38, 95% confidence interval 1.56–3.62, p < 0.001). Among patients with VL suppression, first year VL decay and slope were independent predictors of early CD4 recovery (p = 0.001) and overall gain (p < 0.05). Despite VL suppression, those with slow decay during the first year of HAART as well as during the entire therapy period (overall), in general, gained less CD4 cells compared to the other subjects (133 vs. 195.4 cells/μL; p = 0.001) even after adjusting for potential confounders.

**Conclusions:** In a cohort with free access to healthcare, independent of established predictors of AIDS and CD4 recovery during HAART, cumulative VL and virologic decay patterns were associated with AIDS and distinct aspects of CD4 reconstitution.

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Introduction

The initial goal of highly-active antiretroviral therapy (HAART) was to improve AIDS-free survival and attempt to mitigate the harmful effects of treatment. Immune reconstitution via CD4 recovery served as an intermediate marker for response to HAART because of its predictive capacity for AIDS events and death.[1,2,3] Thereafter, virologic suppression became the
primary target for therapy because it was shown to be an appropriate, early predictor of immunologic response and clinical outcomes.[4,5,6,7] Furthermore, it was demonstrated that incomplete suppression of viral replication allowed for the emergence of drug resistance and ultimately virologic failure.[8,9] These findings led to recommendations in the U.S. Department of Health and Human Services guidelines that patients should achieve complete virologic suppression (viral load [VL] <400 copies/mL by 24 weeks or <50 copies/mL by 48 weeks) and maintain suppression thereafter.[10]

Even among patients reaching these virologic targets, there are significant inter-individual differences in the recovery of CD4+ T cells and risk of clinical events, suggesting that other factors may relate to these outcomes.[11,12,13,14,15,16] Age at HAART initiation, pre-HAART VL and CD4 cell count, magnitude of and time to VL suppression all have been shown to influence CD4 recovery and clinical outcomes. [4,13,17,18,19,20,21,22,23,24,25,26] Although the relationship of virologic decay patterns with VL changes during HAART has been described,[23,27,28,29] the impact of these decay patterns on CD4 reconstitution and risk of subsequent clinical AIDS events has not been fully elucidated. Furthermore, it is also conceivable that the overall VL burden, represented as the cumulative VL during HAART, may also influence CD4 recovery and risk of AIDS events. Hence, we determined whether the patterns of virologic decay and the cumulative VL during HAART were associated with AIDS and CD4 recovery after HAART initiation independent of the currently recommended dichotomous measures of VL suppression[10] within a large, observational cohort with free access to medications and care, high rates of adherence, and low rates of injection drug use.[26,30] If virologic decay measures are independently associated with outcomes, this could provide some explanation as to why some individuals experience inadequate treatment response despite achieving virologic suppression. Additionally, cumulative viral load could serve as a sensitive marker for risk of AIDS after HAART beyond traditional measures.

Materials and Methods

Study Participants

The U.S. Military HIV Natural History Study (NHS) is a prospective multicenter observational study of HIV-infected active duty military personnel and other beneficiaries (spouses, dependents, and retired military personnel) from the Army, Navy/ Marines and Air Force. Seroconverters (SC) were defined as patients having a documented HIV seronegative date prior to the first positive HIV date (see Table S1). The estimated date of seroconversion for SC was defined as the midpoint between the two dates. All CD4 count, VL, and other measurements were done as part of routine clinical care.[31] The clinically-approved methodology for this testing varied by site and over time. Prior ARV use referred to any antiretroviral therapy not meeting the NHS definition of HAART.[26] HAART initiation was the date when HAART was first prescribed.

Ethics Statement

Participants who provided written informed consent and initiated HAART through July 1, 2006 regardless of regimen continuation were included in the present study. The NHS and this substudy have been approved by each center’s Institutional Review Board and the Uniformed Services University of the Health Sciences Institutional Review Board.

Statistical Analysis

VL Parameters. A primary aim of this study was to capture and summarize the overall and early VL dynamics in such a manner as to permit their eventual use in clinical practice. In that regard, we made the following assumptions: i) by the time HAART is typically initiated for an individual in the NHS a natural steady state VL exists; ii) once potent HAART is initiated there is a rapid decline in the VL followed by a slower decline; and iii) such a typical pattern of VL can be explained on the basis of an exponential decay in the circulating VL. The definitions of the parameters used in this study are shown in Table S1, and the theoretical bases for the estimation of these parameters are further described in Note S1. The composite “virologic decay” refers to the application of an exponential decay equation which has been fitted to all viral loads available for an individual after the initiation of HAART. For a majority of participants in this cohort who have a high level of adherence, the virologic decay pattern corresponds to the concatenation of each “classical” (first, second, etc.) phase of decay for that individual. For some participants, their virologic decay does not follow these patterns due to suboptimal adherence, inadequate drug levels, drug resistance, and treatment interruption.

We computed four VL parameters at the level of each individual: (i–ii) exponential decay constant of VL change during entire duration of HAART (overall) and during the first year of HAART, respectively; (iii) VL slope during the first year of HAART obtained using linear Generalized Estimating Equations (GEE) models; and (iv) cumulative VL (Table S1). The VL parameters described above in i, ii, and iii are designated as VL kinetic parameters. Similarly, we computed the following four CD4 count parameters at the level of the individual: (i–ii) slope of the CD4 count change during and after the first two years of HAART; (iii) mean CD4 count after the first two years of HAART; and (iv) overall gain in CD4 counts (Table S1).

Cohort level analyses. The cohort-level analyses made use of all available CD4+ T cell count and VL data for all subjects to generate time-trend lines or curves using linear and non-linear GEE models, assuming an equal correlation structure. The time-trend curves derived by non-linear GEE modeling were refined further using spline smoothed curves with knots at the end of each year since HAART initiation. The resulting curves describe an overall or composite VL pattern for the cohort.

Association analyses. We estimated the parameters detailed in Table S1 for each individual. The association of these individual level parameters with the risk of AIDS (defined using 1993 clinical criteria[32] but did not include a CD4 count <200 cells/μL as an endpoint) was assessed by Poisson regression models, and with recovery of CD4 counts by linear regression models. In these models we accounted for the potential confounding due to VL suppression by HAART by including two covariates - achievement of VL suppression (as defined in Table S1) and the time taken to achieve VL suppression from the start of HAART. As described in the results, we ran these multivariate analyses for the VL parameters that were estimated (i) by including all VL measurements after HAART and separately (ii) by restricting to only those measurements after HAART but prior to the occurrence of the first AIDS event. Statistical significance was evaluated at a type I error rate of 0.05. All statistical analyses were conducted using Stata 7.0 (Stata Corp., College Station, TX).

Results

Cohort-level VL and CD4 changes after HAART initiation

Characteristics of the 2278 participants who initiated HAART are in Table 1. The average follow up time after HAART for
participants was 5.63 years (SD 3.98). Cohort-level non-linear GEE modeling of VL from time-of-HAART initiation in all subjects revealed the following pattern: a precipitous decline in VL during the first year, a temporary rebound at ~1.6 years post-HAART, followed by a relatively steady-state VL thereafter (Fig. 1A). The VL trajectory of subjects who developed AIDS versus those who remained AIDS-free differed significantly as a decline in VL after HAART initiation was not observed in patients who developed AIDS (Fig. 1B). In all subjects (Fig. 1C) and in those who attained VL suppression (Fig. 1D), VL trajectories differed according to the tertiles of the pre-HAART VL such that those who started with higher VLs (upper and middle tertiles of pre-HAART VL) displayed a sharper decline in VL than those subjects categorized to the lower pre-HAART VL tertile (Fig. 1C-D, Table S2).

The cohort-level trajectories in CD4 counts during HAART revealed two phases of CD4 count changes. In phase I, for all subjects initiating HAART there was a rapid increase in CD4 counts during the first two years, followed in phase II by a slower, sustained gain in CD4 cells (Fig. 1A). We stratified the cohort-level changes in CD4 count gains according to whether subjects attained VL suppression (Fig. 1E). This analysis revealed that during the first year of HAART, rapid and similar gains in CD4 counts (~200 cells on average) were observed in those who did (brown curve) or did not (black curve) attain VL suppression (Fig. 1E). However, in contrast to those who attained VL suppression, the initial gains in CD4 counts were not durable among those who did not achieve VL suppression (Fig. 1E).

### Table 1. Characteristics of subjects on HAART studied.

| Characteristic | Median (IQR) or Percentage |
|---------------|---------------------------|
| Age at HAART (y) | 34.27 (29.15–39.61) |
| Female gender | 188 (83.3%) |
| Ethnicity | 2278 |
| European Americans | 1006 (44.2%) |
| African Americans | 1003 (44.9%) |
| Hispanic Americans | 186 (8.2%) |
| Others | 83 (3.6%) |
| Baseline CD4 (cells/µl) | 2284 |
| Nadir CD4 (cells/µl) | 2200 |
| Time from nadir CD4 to HAART initiation (y) | 2200 |
| Baseline VL (log_{10} copies/ml) | 1951 |
| Pre HAART VL (log_{10} copies/ml) | 1979 |
| Overall VL decay constant (x10^{-2}) | 2055 |
| VL decay constant during year one of HAART (x10^{-2}) | 1684 |
| VL slope (log_{10} copies/ml/month) during year one of HAART | 1684 |
| Cumulative VL (log_{10} copies*months/ml) | 1949 |
| Average time to HAART initiation (y) | 2278 |
| Late HAART era | 2278 |
| Prior use of ARV | 2278 |
| AIDS before HAART initiation | 2278 |
| Duration of follow-up on HAART (y) | 2278 |
| VL measurements per individual per year | 2278 |
| CD4 measurements per individual per year | 2278 |
| AIDS after HAART (%) | 2278 |
| VL suppression | 2278 |
| First twelve months | 1722 |
| First six months | 1790 |
| First three months | 1294 |
| CD4 slope in first 2 years after HAART (cells/µl/year) | 1931 |
| CD4 slope after 2 years of HAART (cells/µl/year) | 1532 |
| Mean CD4 count 2 years after HAART (cells/µl) | 1560 |

*Values represent the mean.

Viral Load Decay Modeling
The history of AIDS, nadir CD4, age at HAART initiation and time to VL suppression. The overall decay rate constant was not predictive of rate of CD4 gain in the first 2 years, but was significantly associated with the rate of CD4 gain after two years of HAART, the mean CD4 count two years after HAART, and the overall gain in CD4 cells (Table 3). The decay constant and VL slope in the first year of HAART were mostly predictive of the rate of CD4 cell gain during the first two years and the overall gain in CD4 cells (Table 3). By contrast, the cumulative VL was only predictive of rate of CD4 gains after 2 years and not the overall gain in the CD4 count (Table 3).

The aforementioned data suggested that a slower VL decay during the first year of HAART is associated with both a reduced rate of CD4 gain in the first two years of HAART and overall gain in CD4 cells (Table 3). By contrast, a slower overall VL decay is more predictive of a reduced rate of CD4 gain after 2 years of HAART, lower mean gains in CD4 counts after 2 years of HAART as well as a reduced overall gain in CD4 cells (Table 3). On the basis of these results, we posited that VL suppressors who had a slow VL decay in the first year of HAART and the entire therapy course (overall) would fare the worst with respect to CD4 recovery. To test this, we categorized VL suppressors into two categories.
Table 2. Association of VL parameters with risk of AIDS development after initiation of HAART.

| VL parameter using all available measurements | Unadjusted | | | | | Adjusted | | | | | |
|----------------------------------------------|------------|--|--|------------|--|--|------------|--|--|------------|--|--|
| Overall decay constant                        | 1.32       | 0.96–1.82 | 0.087 | 1.38       | 0.99–1.94 | 0.058       |
| Decay constant in first year                  | 1.03       | 0.72–1.47 | 0.876 | 1.07       | 0.74–1.55 | 0.730       |
| Slope in first year                           | 0.99       | 0.69–1.42 | 0.964 | 1.04       | 0.72–1.51 | 0.828       |
| Cumulative VL                                 | 2.22       | 1.56–3.13 | <0.001| 2.38       | 1.56–3.62 | <0.001       |

| VL parameters by excluding measurements after first AIDS event | Unadjusted | | | | | Adjusted | | | | | |
|---------------------------------------------------------------|------------|--|--|------------|--|--|------------|--|--|------------|--|--|
| Overall decay constant                        | 1.29       | 0.94–1.77 | 0.122 | 1.35       | 0.97–1.89 | 0.080       |
| Decay constant in first year                  | 1.05       | 0.73–1.50 | 0.795 | 1.07       | 0.73–1.55 | 0.732       |
| Slope in first year                           | 1.05       | 0.73–1.49 | 0.805 | 1.05       | 0.72–1.52 | 0.801       |
| Cumulative VL                                 | 2.22       | 1.57–3.13 | <0.001| 2.38       | 1.56–3.62 | <0.001       |

*Results are from a Poisson regression model adjusted for the length of follow-up. Unadjusted results are from the bivariate models with the indicated VL parameter as the predictor and AIDS development as the outcome. All VL parameters were dichotomized based on their respective medians. The RRs are for the association of slow decay (as indicated by less than median decay constants and slope) and high (greater than median) cumulative viral load with AIDS development. Adjusted models are multivariate models that included the following covariates: baseline and nadir CD4 count, pre-HAART VL, time to HAART, age at HAART initiation (per 10 years) and time to VL suppression were included as continuous variables.

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This phenomenon can be seen when a system undergoing small oscillations over time (such as during the dynamic equilibrium of viral load setpoint) undergoes a significant dampening (HAART initiation) and then experiences brief periods of external perturbation (brief treatment interruptions).

Although the importance of early virologic suppression and virologic failure on CD4 recovery has been well-described,[21,22,24,42,43,44] much less is known about the impact of rate of decay or detectable VL after initial suppression of VL on CD4 recovery.[25,38,45,46,47] In this study, we incorporated several of these elements into a single parameter of overall virologic decay. This parameter provides information on the early trajectory as well as the durability of the VL response after the initial decay. We also evaluated cumulative VL because it could be argued that it is the overall exposure to virus that influences CD4 recovery and AIDS.[38,49,50,51] We found that cumulative VL predicted AIDS or death in absence of HAART independent of known risk factors in the Multicenter AIDS Cohort Study.[56] As the number of serious non-AIDS events during HAART increases relative to the number of AIDS events over time, it will be important to determine the association of overall virologic decay and cumulative VL with serious non-AIDS events as has been demonstrated with cancer[57,58] and renal impairment.[59] This data would also suggest that perhaps the cumulative VL even prior to HAART could be associated with clinical events during HAART, supporting the notion that earlier diagnosis and treatment would further reduce the number of these adverse outcomes.

Even among subjects who attained VL suppression, and after adjustment for time to VL suppression, a slow overall VL decay was predictive of late/long-term CD4 changes (rate of CD4 gain, mean CD4 count after two years of HAART, and overall gain in CD4 cells), but not early CD4 changes (rate of CD4 gain during the first two years of HAART). In contrast, a slower VL decay within the first year of HAART associated with early but not later/long-term CD4 changes during HAART. These results suggest that, although among VL suppressors the pace and extent of CD4 gain during the early phases of HAART may be highly correlated with both the early and overall VL decay patterns, durable gains in CD4 cells after two years of HAART may be highly dependent on the overall VL decay pattern. These findings demonstrated that VL suppressors could be stratified into two categories such that those with both a slow early and overall VL decay (slow early/slow overall decay) will achieve CD4 recovery, but the gain in CD4 cells would be significantly muted relative to
all other subjects (Fig. 1F). Because studies have identified polymorphisms that track the durability of CD4 recovery, it will be important to evaluate whether the patterns of VL decay are in part related to such host factors.[11]

The association of the extent of CD4 recovery was strongest with the overall VL decay and not the first year VL decay or the cumulative VL, suggests that these VL parameters may be capturing different aspects of VL changes during HAART (early trajectory and maintenance of suppression). The overall decay provides information throughout the duration of treatment and is not limited to one year of information. Hence, it is probable that the decay pattern occurring after virologic suppression (third phase of VL decay)[60] indexed to the decay patterns that occur immediately after HAART initiation[23] together contribute to the ability of a patient to experience durable immune reconstitution. It remains unclear if the latter phases of immune reconstitution are affected by “blips” or primarily by more substantial viral rebound.[61,62]

In contrast to the overall decay pattern, the cumulative VL is a cause measure of overall VL burden (total virus exposure) during HAART and does not account for VL decay patterns. For example, a patient who suppresses early but has late rebound might have a comparable cumulative VL to that of a patient with predominantly late virologic suppression. This may partly explain why this parameter as computed may not associate strongly with CD4 recovery. However, another explanation hinges on the use of detectable VLs to compute this parameter. Certainly, patients with complete or repetitive virologic rebounds may experience a loss of CD4 recovery; however, the vast majority of patients in this cohort achieved suppression within the first year and the rate of rebound was low.[26] Therefore, at the frequency of available measurements, the cumulative VL may not capture some of the intermittent or ongoing low-level viremia during HAART which may represent actual viral replication in the setting of periodic HAART interruption. Hence, it is conceivable that computation of the cumulative VL using more frequent measurements and/or single copy assays that assess VL below the detectable threshold of commercial assays might reveal that the cumulative VL is a more sensitive marker of not only AIDS risk but also CD4 recovery.

We investigated a large number of prospectively evaluated subjects who have equal access to healthcare and high rates of adherence which in a clinical setting can often be unreliable. We also acknowledge that we studied a total of 52 multivariate models (shown in Tables 2 and 3) and at a global type I error rate of 0.05, 2–3 observed associations are likely to be erroneous. Given the fact, however, that we observed a total of 23 associations to be significant at 0.05 type I error rate, our study results are unlikely to have been influenced by false positive associations due to multiple testing. Finally, although the impact of drug resistance and pharmacokinetic interactions was not examined in this study, prior ARV use was used as a surrogate marker of baseline resistance in the multivariate models.

In summary, our findings underscore that the early and overall patterns of VL decay among VL suppressed patients is an independent determinant of CD4 recovery. In addition, the cumulative VL is a determinant of AIDS risk during HAART. Thus, inter-individual differences in VL decay patterns may partly explain the wide variability in CD4 recovery even among those individuals achieving VL suppression within the recommended timeframe. These results also suggest that regimens that produce the most rapid virologic decay and durable suppression could lead to better clinical/immunologic responses. These parameters could be further developed to enhance clinical trial assessment of ARV regimens and assist clinicians with identifying patients at risk for adverse events beyond standard indicators.

Supporting Information

Table S1 Definitions for various parameters used in this study.

Table S2 Modeling of VL kinetics based on tertiles of pre-HAART VL.

Table S3 Association of VL parameters with risk of AIDS development after initiation of HAART among seroconverters.

Note S1 Statistical concepts in VL parameter estimation.

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Author Contributions

Conceived and designed the experiments: VCM HK SKA JFO MD. Analyzed the data: HK VCM GG JFO. Contributed reagents/materials/analysis tools: HK SKA BA MP. Wrote the paper: VCM HK SKA GG. Gathered clinical data: VCM JFO GW AG NCC ML MD BA IDeCRP HWG. Analysis interpretation and manuscript review: VCM GG JFO GW AG NCC MP ML SKA BA HK.
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