Prognostic Value of Baseline Human Immunodeficiency Virus Type 1 DNA Measurement for Disease Progression in Patients Receiving Nucleoside Therapy

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Human immunodeficiency virus (HIV) type 1 DNA assay data were obtained at baseline from 111 HIV-1–positive subjects who were treated with nucleosides. Higher baseline DNA level, HIV-1 RNA level, and infectious titer were comparably associated with an increased hazard of disease progression (each \( P < .03 \)). Only DNA level was significantly associated with survival (adjusted hazard ratio for 1 log \(_{10}\) higher level, 3.99; 95% confidence interval, 1.44–11.09; \( P = .008 \)).

Measurement of human immunodeficiency virus (HIV) load by, for example, HIV-1 RNA level in plasma, is an essential tool for guiding clinical practice in HIV disease [1]. Underlying the use of assays that measure HIV load are assumptions about their prognostic value for HIV disease progression [2, 3]. Newer HIV load assays must also be evaluated for their prognostic value. One such assay is a prototype developed by Roche Molecular Systems to measure HIV-1 DNA levels in peripheral blood mononuclear cells (PBMC) [4, 5]. This assay measures all forms of intracellular HIV-1 DNA, most of which is proviral DNA.

The AIDS Clinical Trials Group (ACTG) Study 175 was a randomized study of antiretroviral treatment in HIV-1–infected persons monitored for HIV-1 disease progression [6]. A virology substudy measured infectious HIV-1 titer in PBMC and plasma HIV-1 RNA level and determined viral phenotype; these measurements were associated with HIV disease progression [7, 8]. In addition, DNA from PBMC (stored frozen) was isolated, and HIV-1 DNA levels were quantified. The goal of the present study was to evaluate the association of baseline HIV-1 DNA level with baseline and follow-up (weeks 8, 20, and 56) levels of plasma HIV-1 RNA, infectious titer in PBMC, CD4 cell count, and HIV disease progression among the ACTG 175 participants.

Methods. ACTG 175 enrolled 2467 subjects with CD4 cell counts of 200–500 cells/mm\(^3\) to receive, by random assignment, 1 of 4 blinded regimens of antiretroviral therapy; zidovudine (Zdv) alone, Zdv plus didanosine (ddI), Zdv plus zalcitabine, or ddI alone [6]. A subset of 391 subjects was enrolled in the virology substudy [7]. For 354 of the 391 virology substudy subjects, genotypes of human genes for HIV-1 entry coreceptors were obtained to evaluate their association with HIV disease progression [9].

Lysates from frozen PBMC from 141 of these 354 subjects were from samples obtained at baseline and were deemed to be suitable for quantitative HIV-1 DNA analysis. Valid measurements were obtained for 139 subjects (1 tube was missing a draw date; another specimen had no detectable DNA). Of these 139 subjects, 131 also had baseline data on plasma HIV-1 RNA level and determined viral phenotype; these measurements were associated with HIV disease progression [7, 8]. In addition, DNA from PBMC (stored frozen) was isolated, and HIV-1 DNA levels were quantified. The goal of the present study was to evaluate the association of baseline HIV-1 DNA level with baseline and follow-up (weeks 8, 20, and 56) levels of plasma HIV-1 RNA, infectious titer in PBMC, CD4 cell count, and HIV disease progression among the ACTG 175 participants.

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Distributions of baseline variables were described with summary statistics. Between-group baseline quantitative HIV-1
DNA levels were compared by use of the Kruskal-Wallis test. Associations between continuous measures at baseline and during follow-up (weeks 8, 20, and 56) were estimated by Spearman rank correlation coefficient. The HIV-1 DNA levels are expressed as the number of HIV-1 DNA copies in a microgram of total cellular DNA. HIV-1 RNA and DNA levels and infectious titer in PBMC were analyzed on the log scale.

We considered 3 HIV disease progression end points: the ACTG 175 study end point (50% decline in CD4 cell count, AIDS, or death), an AIDS or death end point, and a death (survival) only end point. Distributions of times to events were estimated by the Kaplan-Meier method. Associations between baseline HIV-1 DNA level and disease progression were evaluated by Cox proportional hazards modeling. The approach included univariate modeling of each of the baseline factors for each end point (results not shown), and a multivariate model was obtained by stepwise/forward/backward selection procedures (selection criterion, $P = .05$ on baseline covariates). The baseline HIV-1 DNA level then was added to the resultant model for each end point, to evaluate whether this assay contributed additional information about disease status beyond these baseline covariates.

Results. The 111 subjects who had data available for all relevant measurements were mostly male (88%) and white non-Hispanic (74%), with a median age of 36 years and little antiretroviral treatment experience (64% had <1 week of treatment). Most subjects (81%) had no history of injection drug abuse, 33% had symptomatic HIV disease, and 15% had a syncytium-inducing viral phenotype at study entry. The median baseline HIV-1 DNA levels were 0.78–3.60 log_{10} copies/\text{mg} of DNA in PBMC (median, 2.08 log_{10} copies/\text{mg} of DNA in PBMC; interquartile range, 1.68–2.40 log_{10} copies/\text{mg} of DNA in PBMC). No significant associations between baseline HIV-1 DNA level and most other baseline factors, including the 3 host genotypes (CCR5, CCR2, and stromal cell–derived factor 1; see Methods), were observed, except with disease status and race/ethnicity. Symptomatic subjects had significantly higher levels (median, 2.37 and 1.95 log_{10} copies/\text{mg} of DNA in PBMC) than did nonwhites (median, 1.78; $P = .002$). Baseline HIV-1 DNA level was not significantly associated with CD4 cell count at baseline (Spearman correlation, $-0.14$; $P = .16$) but was significantly associated with baseline plasma HIV-1 RNA level and with baseline HIV-1 titer in PBMC (Spearman correlation, 0.61 for both; $P < .001$) and was significantly associated with follow-up plasma HIV-1 RNA level (Spearman correlations, 0.40–0.54; $P < .001$) and with HIV-1 titer in PBMC (Spearman correlations, 0.41–0.43; $P < .001$) at all 3 time points considered.

Kaplan-Meier plots of disease progression indicate an association between higher (above median) baseline HIV-1 DNA level and more-rapid disease progression (figure 1; $P \leq .01$ for each end point). Cox proportional hazards modeling on the 3 HIV disease–progression end points with respect to baseline plasma HIV-1 RNA level, infectious titer in PBMC, and CD4 cell count were 4.35 log_{10} copies/mL, 1.41 log_{10} IU/10^9 PBMC, and 325 cells/mm³, respectively.

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plasma HIV-1 RNA, HIV-1 titer in PBMC, and HIV-1 DNA level show similar univariate associations among these 3 measurements and the development of an ACTG 175 study end point and AIDS/death end point in terms of the strength of association ($P = .003$, $P = .014$, and $P < .001$ for plasma HIV-1 RNA, HIV-1 titer in PBMC, and HIV-1 DNA level, respectively, for the study end point; $P = .007$, $P = .027$, and $P = .004$, respectively, for the AIDS/death end point; table 1). For the survival end point, the association was significant for HIV-1 DNA level ($P = .007$) but of only marginal significance for plasma HIV-1 RNA level ($P = .05$) and HIV-1 titer in PBMC ($P = .094$).

Multivariate models were built for each end point by using stepwise (and forward and backward) selection procedures for the following baseline covariates: age, sex, race, previous antiretroviral therapy, injection drug abuse, log$_{10}$ plasma HIV-1 RNA level, log$_{10}$ HIV-1 titer in PBMC, CD4 cell count, viral phenotype, and symptomatic status (table 1). For the study and AIDS/death end points, the resultant model contained only baseline plasma HIV-1 RNA level, previous antiretroviral therapy, and viral phenotype; for the survival end point, viral phenotype was the sole significant factor. CD4 cell count and log$_{10}$ HIV-1 titer in PBMC were not selected into any of the models.

When baseline HIV-1 DNA level was added to these models, there was some suggestion that higher levels were associated with a decreased time to developing a study or AIDS/death end point ($P = .09$ and $P = .11$, respectively; table 1). There was a trend for an association of higher HIV-1 DNA level with decreased survival time ($P = .008$), although the addition of both plasma HIV-1 RNA level and previous antiretroviral therapy to this model attenuated this association to be only suggestive ($P = .04$). The adjusted estimated hazard ratios for 1 log$_{10}$ higher baseline HIV-1 DNA level were 2.44 for the study (95% confidence interval [CI], 0.88–6.82), 2.71 for AIDS/death (95% CI, 0.79–9.27), and 3.99 for survival (95% CI, 1.44–11.09) end points.

**Discussion.** Baseline HIV-1 DNA level showed significant associations with other HIV load measurements, as measured by HIV-1 RNA plasma levels and HIV-1 titer in PBMC at baseline and during follow-up. Evidence on whether quantitative HIV-1 DNA provides additional information about the hazard of disease progression, adjusted for other associated factors (e.g., plasma HIV-1 RNA level), was suggestive. Although the event numbers in this exploratory study were small, the data suggest an association of higher baseline HIV-1 DNA level with decreased survival time that warrants further investigation. This association is supported by the observed relationship of increased values of quantitative HIV-1 DNA with symptomatic HIV disease status at study entry.

One hypothesis to explain the observed association is that total body burden of HIV, including the latent reservoir of HIV-1 integrated and quiescent in resting memory CD4 cells, as quantified with this assay, is associated with survival. There is a negative association between HIV-1 DNA copies per CD4 cells and overall CD4 cell count in the absence of suppressive antiretroviral treatment [10]. This may be because the proportion of CD4 cells with integrated HIV-1 DNA increases with time in the absence of effective suppression of HIV replication. Plasma HIV RNA levels reflect recent viral replication and rapidly decrease with effective antiretroviral therapy. However, the
level of replication-competent HIV cultured from PBMC or resting memory CD4 cells may remain stable in many subjects despite effective therapy [11–13]. In other subjects, however, the amount of virus in that latent reservoir may decrease [14]. It is difficult to sample large numbers of patients with the arduous HIV-1 ultrasensitive culture assays of the latent reservoir to assess how this reservoir is associated with long-term HIV disease prognosis. However, this may be more feasible to study with the simpler quantitative HIV-1 DNA assay used here and, if it is associated with measures of replication-competent latent reservoir virus, may allow testing of the pathogenic hypothesis that decreasing the total body burden of HIV, as measured by the total HIV-1 DNA titer in PBMC, may indeed improve survival.

The HIV-1 DNA levels did not have a strong association with CD4 cell count at baseline or during follow-up. Eligible subjects for ACTG 175 were required to have CD4 cell counts within a relatively narrow range of 200–500 cells/mm². In fact, in this sample, 60% of subjects had baseline CD4 cell counts of 13%–24%, making it difficult to observe any true association that may have been observed over a larger range of CD4 cell counts.

HIV-1 titer in PBMC does not seem to add prognostic information when used with HIV-1 RNA, whereas the HIV-1 DNA level in PBMC may do so, as suggested in the multivariate analyses—particularly for survival. The quantitative culture assay for determining HIV-1 titer in PBMC requires viable cells and is performed best with fresh samples. In contrast, the quantitative HIV-1 DNA assay is a biochemical assay that can be done on frozen cell pellets, eliminating the need for viable cells and cell culture. The HIV-1 DNA assay could potentially be done with whole blood or dried blot spots, eliminating the need for cell separation procedures. In addition, DNA polymerase chain reaction does not depend on the efficiency of recovery of RNA from plasma or the activity of extrinsic reverse transcriptase. The latter may be inhibited by anticoagulants, hemoglobin, and other plasma components [15]. Thus, the relatively easier sample preparation and assay performance make the quantitative HIV-1 DNA assay attractive in resource-constrained clinic settings or in clinics without on-site laboratories. Measurement of HIV-1 DNA in PBMC from frozen samples or dried blot spots may provide a candidate assay that is a more robust, lower cost alternative to the reservoir to assess how this reservoir is associated with long-term HIV disease prognosis. However, this may be more feasible to study with the simpler quantitative HIV-1 DNA assay used here and, if it is associated with measures of replication-competent latent reservoir virus, may allow testing of the pathogenic hypothesis that decreasing the total body burden of HIV, as measured by the total HIV-1 DNA titer in PBMC, may indeed improve survival.

In summary, levels of HIV-1 DNA in PBMC are associated with other measures of virus load and disease progression. Thus, quantitative HIV-1 DNA measurement might help predict the clinical outcome of persons infected with HIV and may be a useful marker for clinical trial evaluation. The association of HIV-1 DNA level in PBMC with survival also suggests a testable pathophysiologic hypothesis: the total magnitude of the reservoir of HIV may be associated with survival.

ACTG 175 Virology Team

Study team members were as follows: D. Katzenstein (Stanford University, Palo Alto, CA), S. Hammer (Columbia University College of Physicians and Surgeons, New York, NY), J. Lathey (University of California at San Diego), S. Fiscus (University of North Carolina at Chapel Hill), B. Jackson and H. Farzadegan (Johns Hopkins University, Baltimore, MD), S. Rasheed (University of Southern California, Los Angeles), T. Elbeik (University of California at San Francisco), R. Reichman (University of Rochester, Rochester, NY), A. Japour (Beth Israel Deaconess Medical Center, Boston, MA), R. D’Aquila (Massachusetts General Hospital and Harvard Medical School, Boston), W. Scott (University of Miami, Florida), and B. Griffith (Yale University, New Haven, CT).

References