Higher Baseline Levels of Plasma Human Immunodeficiency Virus Type 1 RNA Are Associated with Increased Mortality after Initiation of Triple-Drug Antiretroviral Therapy

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We evaluated 1422 antiretroviral therapy (ART)–naive patients infected with human immunodeficiency virus (HIV) who initiated highly active antiretroviral therapy between 1 August 1996 and 31 July 2000 and were monitored until 31 March 2002. Patients were stratified on the basis of baseline levels of HIV RNA (<50,000, 50,000–99,999, and ≥100,000 copies/mL). Cox regression was used to determine independent predictors of time to death. After adjustment for adherence to ART and other potential confounders, baseline levels of HIV RNA of ≥100,000 copies/mL remained independently associated with mortality (adjusted relative hazard, 1.71; 95% confidence interval, 1.08–2.70; P = .023). If appropriately confirmed, these findings have important implications for the development of therapeutic guidelines.

Cohort studies of patients infected with human immunodeficiency virus (HIV)–1 conducted before the era of highly active antiretroviral therapy (HAART) have demonstrated that the level of plasma HIV-1 RNA is an independent predictor of progression to HIV disease [1]. In the HAART era, however, there is a great deal of uncertainty regarding the prognostic value of baseline levels of HIV RNA among treated patients. This has led to substantial discrepancy between expert recommendations, with regard to the initiation of antiretroviral therapy (ART) [2].

A recent study has described the rates of progression to HIV disease, stratified by baseline CD4 cell counts and baselines levels of HIV RNA, among ART-naive patients receiving HAART (median follow-up, 28 months) [3], and found that baseline levels of HIV RNA were not independently associated with survival, a finding that was confirmed by several other investigators [4]. In contrast, a more recent international collaboration reported that having a baseline level of HIV-1 RNA of ≥100,000 copies/mL was independently associated with all-cause mortality [5]. A limitation of these and other population-based analyses [3, 5] has been that they have not been adjusted for the patients’ adherence to the ART regimen. This is critically important because incomplete adherence has been shown to be associated with increased mortality [6]. As such, the association between baseline levels of HIV RNA and mortality may simply be driven by nonadherent patients, among whom levels of HIV RNA would likely be among the strongest determinants of progression to HIV disease [1]. In the present study, we sought to investigate the possible association between baseline levels of HIV RNA and mortality, after adjustment for...
other known prognostic variables, including patients’ adherence to ART, baseline CD4 cell counts, and baseline AIDS diagnoses.

**PATIENTS, MATERIALS, AND METHODS**

The distribution of antiretroviral medications in British Columbia, Canada, has been described in detail elsewhere [3, 6]. In brief, the BC Centre for Excellence in HIV/AIDS Drug Treatment Program remains the only free source of antiretroviral medications in the province. In June 1996, the Centre adopted plasma virus load–driven ART guidelines consistent with those put forward by the International AIDS Society—USA [7]. In brief, ART-naive patients with plasma levels of HIV-1 RNA of >100,000 copies/mL were offered triple-drug regimens (i.e., 2 nucleosides plus either a protease inhibitor or a nonnucleoside reverse transcriptase [RT] inhibitor), and those with plasma levels of HIV-1 RNA of 5000–100,000 copies/mL were offered dual-nucleoside therapy. Consistent with contemporary practice, in July 1997, the Centre guidelines were revised to recommend triple-drug combination therapy for all ART-naive patients with plasma levels of HIV-1 RNA of >5000 copies/mL or CD4 cell counts of <500 cells/μL. Plasma virus loads were measured by the Amplicor HIV-1 Monitor (Roche Diagnostics). For all program participants, a complete prospective profile of ART is maintained, including the medications prescribed, the dose, the dispensation dates, and the quantity dispensed.

In the present study, analyses were restricted to HIV-infected men and women who had been ART-naive and who were first prescribed triple-drug ART between 1 August 1996 and 31 July 2000. Study subjects were initially prescribed triple-drug combination therapy, with regimens including 2 nucleoside RT inhibitors and either a protease inhibitor or a nonnucleoside RT inhibitor, at the discretion of the enrolling physician. For the purposes of the present study, we followed the intent-to-treat principle, and subjects were included because they were first dispensed antiretroviral drugs, regardless of whether their therapeutic regimen was later modified.

We evaluated the time to death after the initiation of HAART. Deaths occurring during the follow-up period were identified on a continuous basis from physician reports and by record linkages performed with the British Columbia Division of Vital Statistics. To be consistent with earlier analyses [3], deaths from non–HIV-related causes, suicides, and overdoses of illicit drugs were censored at the time of death and were classified as “nonevents” in the primary analysis. All-cause mortality was evaluated in a subanalysis.

We evaluated the cumulative mortality rates, by Kaplan-Meier methods, and Cox proportional-hazard regression was used to calculate the univariate and adjusted relative hazard (RH) [8]. The assumption of proportional hazard was validated by inspection of log (−log [survival function]) estimates against log time plots. To evaluate the effect of baseline virological and immunological status, we stratified patients into low (<50,000 copies/mL), medium (50,000–99,999 copies/mL), and high (≥100,000 copies/mL) HIV RNA strata, and low (<50 cells/μL), medium (50–199 cells/μL), and high (≥200 cells/μL) CD4 cell count strata. These categories were a priori selected on the basis of the several recent studies and therapeutic guidelines that have highlighted the clinical significance of these cutoff values [2, 3, 5]. We further stratified patients into “adherent” and “nonadherent” categories on the basis of prescription-refill compliance [9].

The definition of adherence was based on the ratio of time that dispensed medication would last, as a proportion of follow-up time during the first year of therapy. Previous studies have demonstrated how this estimate strongly predicts virological response and mortality and how it can adjust for the potentially confounding effect of treatment interruption [6, 10]. Patients were a priori defined as nonadherent if they received antiretroviral medications <95% of the time, on the basis of previously published work [10]. Additional variables examined in the present study include the following: protease inhibitor use in the initial regimen (yes vs. no); a previous diagnosis of AIDS (yes vs. no); age, sex, and physician experience (≥6 patients previously enrolled in the program) [6]; and date of initiation of therapy (before or after July 1997) [11]. All multivariate models described here were fit by use of the same protocol of adjusting for all variables that were statistically significant (P < .05) in univariate analyses. All statistical analyses were performed by SAS software (version 6.0). All tests of significance were 2-sided, and P < .05 was considered to be statistically significant.

**RESULTS**

Between 1 August 1996 and 31 July 2000, a total of 1583 ART-naive patients, ≥18 years old, began triple-drug combination therapy. Of these, 161 (10.2%) were excluded for not having both a baseline CD4 cell count and a plasma HIV-1 RNA level available within 6 months before the initiation of ART; therefore, the study sample was based on 1422 (89.8%) subjects (1198 [84.2%] men and 224 [15.8%] women). No differences in sex, baseline AIDS status, and subsequent mortality was observed between the study sample and those excluded. However, persons excluded from this analysis were more likely to be younger (P = .04) and receiving protease inhibitors (P = .02).

The overall median follow-up time was 40.1 months (interquartile range [IQR], 27.7–52.9 months). At baseline, the median age of participants was 37.2 years (IQR, 32.2–43.7 years); the median CD4 cell count was 270 cells/mm³ (IQR, 130–420 cells/mm³); and the median plasma HIV RNA level was 120,000 copies/mL (IQR, 38,000–300,000 copies/mL). Overall, 983 pa-
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Figure 1. Kaplan-Meier product-limit cumulative survival estimates stratified by the low (<50,000 copies/mL), medium (50,000–99,999 copies/mL), and high (≥100,000 copies/mL) baseline human immunodeficiency virus (HIV) RNA strata. The diminishing number of patients at risk at each subsequent interval is the result of events or limited follow-up. ART, antiretroviral therapy.

Table 1 shows the univariate and adjusted RH of mortality. As shown here, the above finding was consistent by Cox regression analyses, with only the high HIV RNA strata being associated with death (adjusted RH, 1.71; 95% confidence interval [CI], 1.08–2.70; P = .023) after adjustment for all relevant covariates, including adherence (adjusted RH, 0.34; 95% CI, 0.25–0.48; P < .001) and CD4 cell count. When all-cause mortality was considered, baseline levels of HIV RNA of ≥100,000 copies/mL remained independently associated with death when the above analyses were repeated. No statistically significant interactions were found, and no interactions were entered into the final model because they did not improve the model’s fit.

DISCUSSION

The present study has demonstrated that patients with baseline levels of HIV RNA of ≥100,000 copies/mL are at increased risk of death after the initiation of HAART, compared with those with baseline levels of HIV RNA of <100,000 copies/mL, even after adjustment for adherence. There was no evidence of a difference in the time to death between patients in the <50,000 and those in the 50,000–99,999 copies/mL baseline HIV RNA strata.

The observation that baseline levels of HIV RNA of ≥100,000 copies/mL were associated with mortality after the initiation of HAART will add to the debate over when ART should best be initiated. One possible clinical strategy to address this concern would be to stage patients for earlier ART, before levels of HIV RNA increase to >100,000 copies/mL. However, as noted by others, among untreated patients, levels of HIV RNA increase gradually and nonuniformly [12], and earlier initiation of treatment would place patients at increased risk of adverse effects, toxicity, and premature evolution of resistance [13]. An alternative strategy that should ideally be explored through pro-
spective clinical trials would be to stage patients who have higher levels of HIV RNA for more-potent initial antiretroviral regimens [14]. It is noteworthy that the association between baseline levels of HIV RNA of \( \geq 100,000 \) copies/mL and higher mortality in the present study differs from an earlier analysis of a cohort of patients who received only 27.7 months of follow-up [3]. The statistical difference has emerged as a result of the longer follow-up that the patients in the present study received, which was 40.1 months. Further study will be required to discern why patients with high levels of HIV RNA may experience increased levels of progression to HIV disease after the initiation of HAART. Additional studies should attempt to discern the relative effect of patients’ adherence to ART, baseline CD4 cell counts, and plasma levels of HIV RNA on virological responses. This is critical because, if differential virological responses among patients with baseline levels of HIV RNA of \( \geq 100,000 \) copies/mL explain the association between baseline levels of HIV RNA and mortality [4], it has implications for the revision of therapeutic guidelines regarding the selection of the initial ART regimen [14].

It is important to stress that all patients in the present study were ART naive before initiating HAART and that the data were derived in a setting where all HIV/AIDS care, antiretroviral drugs, and laboratory monitoring are available free of charge and where previous studies have shown that virtually all patients acquire antiretroviral drugs through a centralized source. Also, the centralized death registry enabled complete population-level data on HIV/AIDS deaths for the entire province. Because a complete prospective record of antiretroviral-drug dispensation was maintained, it was possible to precisely determine each individual’s level of treatment received. Although previous studies have demonstrated that measuring daily adherence to ART can be fraught with difficulties that may over- or underestimate a patient’s actual exposure to treatment, the use of refill compliance as a surrogate for adherence has been validated elsewhere [6, 9, 10], and it has previously been shown that adherence measured during the first year predicts mortality when the data are left censored and the 1-year point is treated as time zero [15]. Nevertheless, prescription-refill compliance only provides an estimate of actual daily adherence. Finally, newer antiretroviral agents, such as those containing ritonavir-boosted protease inhibitors, abacavir, or tenofovir, are not well represented with long duration of follow-up, and the present study was restricted to patients who initiated a HAART regimen with a maximum of 3 antiretroviral agents.

In summary, we found that mortality increased among patients with baseline levels of HIV RNA of \( \geq 100,000 \) copies/mL. There was no evidence of a difference in the time to death between patients in the \( <50,000 \) and those in the \( 50,000–99,999 \) copies/mL baseline HIV RNA strata. The association between high levels of HIV RNA and mortality persisted after adjustment for adherence, suggesting that the association is not simply driven by more-rapid progression to HIV disease among nonadherent patients, among whom levels of HIV RNA would be expected to be among the most dominant predictors of progression to HIV disease [1]. If appropriately confirmed, these findings have important implications for the development of therapeutic guidelines.

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### Table 1. Univariate and multivariate Cox proportional-hazard analyses of the time to death, among 1422 antiretroviral therapy–naive patients initiating highly active antiretroviral therapy (HAART) between 1 August 1996 and 31 July 2000.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted RH</th>
<th>Adjusted RH&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td>RH 95% CI</td>
<td>P</td>
</tr>
<tr>
<td>Adherence, &gt;95% vs. &lt;95%</td>
<td>0.42 0.31–0.58</td>
<td>&lt;.001</td>
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<tr>
<td>CD4 count, cells/µL</td>
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<tr>
<td>&gt;200</td>
<td>1.00</td>
<td>…</td>
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<tr>
<td>50–199</td>
<td>2.98 2.07–4.30</td>
<td>&lt;.001</td>
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<tr>
<td>&lt;50</td>
<td>4.44 2.95–6.70</td>
<td>&lt;.001</td>
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<tr>
<td>HIV-1 RNA, copies/mL</td>
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<td></td>
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<tr>
<td>&lt;50,000</td>
<td>1.00</td>
<td>…</td>
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<tr>
<td>50,000–99,999</td>
<td>1.32 0.70–2.48</td>
<td>.392</td>
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<tr>
<td>&gt;100,000</td>
<td>2.43 1.57–3.78</td>
<td>&lt;.001</td>
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**NOTE.** CI, confidence interval; RH, relative hazard.

<sup>a</sup> The model was also adjusted for protease inhibitor vs. nonnucleoside reverse transcriptase inhibitor, in the initial regimen, physician experience, age, and AIDS at baseline. Physician experience and age remained

\( P<.05 \) in multivariate analyses. The no. of patients in each HIV RNA strata is shown in figure 1.
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