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The relationship between antiretroviral drug concentrations and persistent low-level viremia among HIV-infected women in the United States.

By

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The relationship between antiretroviral drug concentrations and persistent low-level viremia among HIV-infected women in the United States

By

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B.A., Rice University, 1999

M.D., Johns Hopkins University School of Medicine, 2003

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Abstract

The relationship between antiretroviral drug concentrations and persistent low-level viremia among HIV-infected women in the United States.

By Anandi N. Sheth

Background: While most HIV-infected patients receiving combination antiretroviral therapy (cART) achieve plasma HIV RNA level (viral load, VL) below the assay limit of detection, some patients experience episodes of low-level viremia (LLV) with detectable VL <1000 copies/ml. Persistent LLV is associated with cART drug resistance and regimen failure; its cause remains unclear, but could be due to inadequate antiretroviral (ARV) concentrations. The levels of ARVs in hair were previously shown to predict virologic success. We estimated the prevalence of persistent LLV in a cohort of HIV-infected women receiving cART and evaluated the relationship between persistent LLV and hair ARV concentrations.

Methods: 1320 HIV-infected women enrolled in the Women's Interagency HIV Study who reported ARV use for at least 1 year and achieved plasma VL <1000 copies/mL were classified into one of four virologic outcome categories: 1) virologic failure (single VL ≥1000 copies/ml), 2) persistent LLV (≥2 consecutive detectable VL <1000 copies/ml), 3) intermittent LLV (<2 consecutive detectable VL <1000 copies/ml), or 4) sustained virologic suppression (undetectable VL for all visits). 797 women had at least one hair ARV concentration measurement during the follow-up period. We used multivariable logistic regression models to evaluate the relationship between hair ARV concentrations and persistent LLV.

Results: Sustained virologic suppression, intermittent LLV, persistent LLV and virologic failure occurred in 31%, 26%, 14% and 29% of participants, respectively. Participants with virologic failure reported lower adherence, started cART earlier, were more likely to receive protease-inhibitor-based cART, and were more likely to have hair ARV concentrations in the lowest quartile. Only receipt of protease-inhibitor-based cART was significantly associated with persistent LLV (compared with intermittent LLV or viral suppression). In a multivariable logistic regression model, hair ARV concentrations did not significantly differ between women with persistent LLV versus intermittent LLV/ viral suppression.

Conclusions: Virologic outcomes for this large cohort of HIV-infected women were suboptimal, with almost half of participants experiencing either persistent LLV or virologic failure. Hair ARV concentrations were not associated with persistent LLV, suggesting that ongoing viremia arises independently of ARV exposure. Future research is needed to elucidate the pathogenesis of persistent LLV to improve cART outcomes.

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INTRODUCTION

Under current guidelines for the treatment of human immunodeficiency virus (HIV) infection, the goal of combination antiretroviral therapy (cART) is to achieve plasma HIV RNA (viral load, VL) suppression below the limits of detection of standard commercial assays within 6 months (1, 2). However, many patients on cART either intermittently or persistently have detectable plasma virus at low levels below 1000 copies/mL. Intermittent episodes of low-level detectable viremia, or "blips," are common in HIV-infected patients on cART, have not been associated with plasma antiretroviral drug concentrations, drug resistance mutations, or poor clinical outcomes, and are thought to be due to either laboratory artifact or random statistical variation (3). However, patients with either persistent episodes of low-level viremia (LLV) have been found to have increased risk of immune activation (4), genetic resistance mutations (5) and virologic failure (4, 6-11).

While increasing evidence has demonstrated the negative long-term effects of persistent LLV, its etiology remains controversial. Two hypotheses exist regarding the source of plasma HIV RNA in patients with LLV, which are not mutually exclusive: 1) virus originates from ongoing active viral replication in the blood; and 2) virus originates from reservoirs of latently-infected cells. The first hypothesis implies that cART does not fully suppress plasma virus replication amongst individuals with LLV (12), presumably due to inadequate drug exposure, which may be caused by suboptimal adherence, poor absorption, food or drug interactions, or drug metabolism issues.

In order to determine whether persistent LLV is indeed related to inadequate antiretroviral (ARV) drug exposure, an accurate measure of drug exposure is needed.

Direct measurement of ARV drug concentrations, while not widely available for clinical use, provides an objective assessment of drug exposure compared with self-reported or other adherence measurements. An association between persistent LLV and drug concentrations would support the hypothesis that ongoing HIV replication persists due to inadequate ARV exposure. Finding an association between ARV exposure and low-level viremia could inform interventions to enhance the suppressive ability of cART through increased drug exposure, resulting in improved clinical outcomes in patients with LLV. However, single plasma levels of ARVs are highly variable (13) and reflect only a short duration of drug exposure. Work from the Women's Interagency HIV Study (WIHS) has demonstrated that ARV concentrations in small hair samples reflect drug uptake over weeks to months (14) and are stronger predictors of treatment success (i.e., virologic suppression) than self-reported adherence or plasma ARV levels (15, 16).

In this study, we estimate the prevalence of LLV and other virologic outcomes using data from a large, observational cohort of HIV-infected women in the United States. Since previous studies of LLV and HIV reservoirs have occurred almost exclusively in men, we have focused on women, who comprise nearly one-quarter of HIV-infected individuals in the United States, for this analysis. We then evaluated factors associated with persistent LLV among HIV-infected women on cART, focusing on the relationship between hair antiretroviral drug concentrations and persistent LLV (Figure 1). We hypothesized that HIV-infected women with persistent LLV have lower hair antiretroviral drug concentrations than women with sustained viral suppression or intermittent LLV.

BACKGROUND

Nearly 1.2 million people were living with HIV in the United States by the end of 2012, and nearly one-quarter were women (17, 18). In addition, 50,000 new HIV infections occur annually in the United States (17), a number that has been steady despite overall improvements in HIV care and increased options for HIV prevention. HIV infects the immune cells (specifically, CD4+ T lymphocytes) and subsequently either integrates into host DNA (latency) or replicates in activated cells, releasing more virus into the blood, and causing host cell death. Antiretroviral therapy interrupts this cycle, thereby inhibiting HIV replication, suppressing plasma HIV RNA to below the limit of detection, thereby allowing for immune recovery and decreased infections, morbidity, and mortality. However, despite ART, latently infected cells remain in a number of sites, including the blood, bone marrow, lymph nodes, and gut.

While most HIV-infected patients on cART achieve plasma VL suppression below the limits of detection of standard commercial assays within 6 months, some patients on cART experience persistent LLV, which describes LLV that persists across successive VL measurements (4-8). The prevalence of persistent LLV has been estimated between 1.7 – 12% from studies occurring mostly in white and male populations (6, 8-10). Persistent LLV has been associated with development of drug resistance mutations and virologic failure (4-11, 19, 21). There is also concern that LLV may increase levels of inflammation and immune activation, with long-term implications for cardiovascular and other “non-AIDS related” morbidity and mortality in this population (21, 22).

Despite the adverse clinical outcomes associated with persistent LLV, the etiology of this condition is poorly understood (23). Persistent LLV is likely caused by

ongoing viral replication in virus reservoirs or latently infected cells, and cART may be inadequate to suppress the sources of viremia in these patients (24). However, the role of ARV exposure in the development of LLV is unclear. Antiretroviral therapy may be incompletely suppressive due to suboptimal adherence, and some studies have examined the relationship between adherence and LLV. However, such studies have yielded conflicting results, reflecting the heterogeneity and inaccuracy of traditional adherence assessments (25). One study utilizing electronic adherence measurements found a significant association between decreased adherence and transient elevations in plasma viral load (26). In contrast, other studies measuring adherence with electronic measures, self-report and pharmacy refills did not find significant associations between adherence and low-level viremia (3, 27, 28). However, the lack of association observed in some studies may reflect the limitations of self-reported adherence as an accurate measure of actual drug intake (25). In addition, ARV exposure in the setting of optimal adherence may still be compromised by idiosyncratic pharmacokinetics, drug or food interactions, or insufficient penetration of the drugs in tissue reservoirs.

Direct measurement of ARV drug concentrations, while not widely available for clinical use, provides more objective assessment of drug exposure than self-reported or electronic adherence measurements (29, 30). Two prior studies have investigated plasma drug concentrations and low-level viremia and not found an association. The first examined plasma ARV (nelfinavir, ritonavir, efavirenz, lopinavir, and saquinavir) concentrations in 10 patients during episodes of transient viremia >50 copies/mL, and did not find a temporal association between detectable viremia and drug concentration (3). The second found no difference in plasma efavirenz levels among those with

undetectable viral loads versus those whose viral loads which were detectable but less than 50 copies/mL (28). However, both studies included patients with intermittent rather than persistent viremia, which has different long-term consequences and may have a different pathophysiologic basis.

Missing from the therapeutic landscape are accurate, cost-effective measures of antiretroviral exposure that can predict the full spectrum of virologic outcomes, from persistent LLV to virologic failure. Therapeutic drug monitoring of plasma antiretroviral levels has been inconsistently associated with outcomes on treatment (31). Furthermore, drug levels demonstrate significant intra-individual variability that limit utility (13) and reflect only a short duration of drug exposure.

Combination ART most often consists of a three drug regimen consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) and a third "anchor" drug, usually either a protease inhibitor (PI), non-nucleoside reverse transcriptase inhibitor (NNRTI), or integrase inhibitor. Work from the WIHS has shown that levels of anchor ARV drugs in hair specimens are robust predictors of virologic response to cART (15, 16). Hair levels have been shown to be stronger predictors of treatment success (i.e., viral suppression) than self-reported adherence of plasma ARV levels (15, 16). Hair ARV levels provide a non-invasive measure of long-term (weeks to months) exposure to antiretrovirals, analogous to hemoglobin A1C for blood glucose measurement (14). Hair ARV levels could someday guide intensified adherence interventions or cART regimen modification in patients at risk for treatment failure. Hair sampling also provides a means to address both adherence and pharmacokinetics in viremic patients when viral resistance testing, which often cannot be performed in the low-level VL range, is not possible. This

may be of particular interest in resource-limited settings, where routine VL or genetic resistance assays are unavailable or impractical (32). Thus hair ARV levels offer an opportunity to evaluate antiretroviral exposure in individuals with suboptimal responses to cART.

Finally, certain populations may be more at risk for virologic failure and genetic resistance than others. HIV-infected women, for instance, were more likely to experience virologic failure than men in one study of persistent LLV (8). Previous literature has pointed to multiple factors that may disadvantage HIV-infected women on cART as compared to men, including higher rates of cART discontinuation (33), lower rates of tolerability (34) and distinct drug pharmacokinetics and/or toxicities (35). However, most studies on LLV have been conducted in predominantly male cohorts, and studies of HIV reservoirs have occurred almost exclusively in men, leaving the questions of how frequently, to what degree, and with what associated factors this particular virologic outcome occurs in HIV-infected women on cART largely unanswered.

METHODS

Hypothesis and specific aims

This analysis was conducted with the following two specific aims:

1) Aim 1: To estimate the prevalence of persistent LLV and other virologic outcomes in HIV-infected women on cART.
2) Aim 2: To assess the relationship between antiretroviral drug exposures, using hair drug concentrations as a marker, and persistent LLV among HIV-infected women on cART, controlling for potential confounders.

Our overall hypothesis was that HIV-infected women with persistent low-level detectable HIV RNA levels have lower hair antiretroviral drug concentrations than women with sustained viral suppression or intermittent low-level detectable HIV RNA levels.

Study design

We conducted a retrospective analysis of data from the Women's Interagency HIV Study (WIHS), the largest observational cohort of HIV-infected women and sero-negative women at risk of HIV infection in the United States (36, 37). As of March 2014, 4,346 participants have been enrolled in ten clinical sites in the United States, including the six original sites included in this analysis, located in Brooklyn, NY; Bronx, NY; Los Angeles, CA; San Francisco, CA; Chicago, IL; and Washington, DC. Since the beginning of WIHS in 1994, enrolled women participate in study visits every 6 months. Sociodemographic and clinical data are collected at these visits through structured interviews and physical examinations by trained staff. Blood samples are also collected for HIV RNA and CD4 cell count measurements. HIV RNA measurements are performed by a single reference laboratory for all sites. Since 2003, small hair samples

(~10-20 strands) have been collected at biannual visits for drug concentration measurements of the anchor antitretroviral drug, also performed at a single reference laboratory.

WIHS protocols and informed consent materials were reviewed and approved by institutional review boards at all participating institutions, including Emory University. This study was reviewed and approved by the WIHS executive committee, which consists of investigators from each site. The dataset used for this analysis was provided by the WIHS Data Management & Analysis Center (WDMAC) based in Baltimore, MD, and lacked any protected health information.

Study population

This study analyzed data collected from HIV-infected WIHS participants from 2003 through 2012. We included WIHS participants who met the following inclusion criteria: 1) HIV-infected, 2) Reported taking ARVs for at least 1 year, 3) had VL data available from 2003 to 2012 for at least 2 consecutive biannual study visits, and 4) had achieved plasma VL < 1000 copies/mL during at least 1 study visit. For all analyses involving hair ARV concentrations, we included only women who met the above criteria and also had at least 1 hair drug concentration measurement available. We defined a follow-up period to be included for analysis for each participant who met these inclusion criteria. This follow-up period began at the first study visit when plasma VL <1000 copies/mL. The follow-up period ended when either 1) a visit with VL ≥ 1000 copies/mL occurred, 2) the visit prior to ARV discontinuation, 3) the visit prior to missing ARV use data and detectable or missing VL, or 4) the last recorded visit in WIHS as of September 30, 2013.

Measurements

VL outcome categories: Using the pattern of VL measurements for each participant over their analytic follow-up period, women were classified into one of four VL outcome categories. If a participant met criteria for more than one category, she was categorized in the following order of preference: (1) virologic failure: at least 1 VL ≥ 1000 copies/mL; (2) persistent LLV: ≥2 consecutive detectable VL <1000 copies/ml; (3) intermittent LLV: <2 consecutive detectable VL <1000 copies/ml, (4) sustained viral suppression: undetectable VL for all study visits. The primary outcome of interest was persistent LLV compared with either intermittent LLV or sustained viral suppression.

Hair ARV mesurements: The primary exposure of interest was ARV concentration of the anchor drug. Hair samples (~10-20 strands, or 1-3 mg) have been collected from the occipital region of the scalp from HIV-infected participants enrolled in WIHS since 2003. From 2003 to 2011, hair samples were collected only from HIV-infected women reporting ARV use in the past 4 weeks prior to the study visit. Beyond 2011, hair specimens were collected from all HIV-positive women, regardless of ARV use, but only hair ARV data from women reporting ARV use were used in this analysis. Hair specimens were then sent to the San Francisco WIHS group, which has developed and validated assays for measuring ARV concentrations in small hair samples by liquid chromatography coupled with tandem mass spectrometry, as previously described (38, 39). Hair levels of atazanavir (ATV), darunavir (DRV), lopinavir (LPV), nevirapine (NVP), efavirenz (EFV) and raltegravir (RAL) were measured in hair specimens obtained at study visits at which participants reported use of one or more of these ARVs. The assays for ATV, LPV, NVP and EFV from small hair samples have been validated for the

following ranges, and only values that fell within these ranges were used in this analysis: for ATV, LPV and EFV, the validated range is 0.05-20 ng/mg hair; for NVP, the validated range is 0.25-100 ng/mg hair. Of note, validated ranges for DRV and RAL hair levels have not yet been published, thus any value obtained was used for analysis

In order to be able to pool hair concentrations across drugs, we first divided hair concentrations obtained across all person-visits for each drug into quartiles. Second, for each participant, the nadir hair concentration measured during the analytic follow-up period was selected. The rationale for using the nadir hair concentration over the entire follow-up period is that this concentration most closely represents the "worst case scenario" in terms of drug exposure for each participant. Third, the quartile of the nadir concentration was determined for each participant and used for participant-level analyses. If more than 1 ARV was measured at the same time, the participant's hair level was categorized as the highest quartile of all of the measured ARVs.

Additional covariates: Other covariates included in this analysis included age, race/ethnicity, pre-cART VL, pre-cART CD4 cell count, self-reported medication adherence in the last 6 months, cART regimen type, and year of cART initiation. Values recorded at the start of the analytic follow-up period were used in this analysis. In addition, the duration of analytic follow-up period was also evaluated as a covariate.

Pre-cART VL and CD4 cell count were obtained retrospectively through medical record abstraction. The lower limit of detection (LLD) of VL assays varied throughout the study period from 20-400 copies/mL, but was < 80 copies/mL for >99% of all person-visits included in our analysis. Data on ARV use and adherence were obtained by patient self-report at every study visit. ARV use was recorded in terms of the specific ARV or

combination pill. Of note, WIHS participants were encouraged to bring their medication bottles to study visits for verification purposes. Adherence was assessed on a categorical scale from 0-100% in the previous 6 months.

Antiretroviral regimens were classified into 4 major categories: (1) Non-nucleoside reverse transcriptase inhibitor (NNRTI)-based cART: ≥2 nucleoside reverse transcriptase inhibitors (NRTIs) and ≥1 NNRTI; (2) protease inhibitor (PI)-based cART: ≥1 NRTI and ≥1 PI or ≥2 PIs; (3) integrase inhibitor-based cART: ≥2 NRTI and ≥1 integrase inhibitor; or (4) NRTI-only containing cART: ≥3 NRTIs. Any other regimen types were not included in analyses of regimen types. Year of cART initiation was divided into the following periods: 1995-1999 (corresponding to early cART regimens and heavy previous use of mono- and dual therapies); 2000-2003 (corresponding to second-generation PIs and use of the NRTI tenofovir); 2004-2008 (corresponding to the availability of single-tablet/fixed dose cART regimens and increased tolerability of regimens); and the period after 2008 (corresponding to increased use of the newer PIs, integrase inhibitors, and greater single-coformulated tablet regimen use [40]).

Statistical analysis

For Aim 1, the number of participants in each VL outcome category was used to estimate the prevalence (and 95% confidence interval [CI]) of virologic suppression, intermittent LLV, persistent LLV and virologic failure. Summary statistics were performed for all exposure variables of interest (age, race/ethnicity, pre-cART VL, pre-cART CD4 cell count, self-reported medication adherence in the last 6 months, cART regimen type, and year of cART initiation), both overall and by VL outcome category. Exposure variables were compared by VL outcome category using either chi-square or

Fisher's exact tests for categorical exposure variables, or two-sample t-tests, Wilcoxan rank sum tests, or one-way analysis of variance for continuous exposure variables.

For Aim 2, we focused on the outcome of interest, persistent LLV and thus dichotomized VL outcome categories in the following way. Participants in the virologic failure category were excluded, and the participants with persistent LLV were compared to those with either intermittent LLV or sustained viral suppression (persistent LLV = 1, intermittent LLV or viral suppression = 0). The intermittent LLV and sustained viral suppression categories were grouped because these are the categories that are not associated with adverse clinical outcomes.

We then performed bivariate analysis between exposure variables (age, race/ethnicity, pre-cART VL, pre-cART CD4 cell count, self-reported medication adherence in the last 6 months, cART regimen type, and year of cART initiation) and the outcome of interest (persistent LLV) using separate logistic regression models for each exposure variable and calculate an unadjusted odds ratio (OR) for each exposure variable to estimate its association with persistent LLV:

$$\text{Model: } \text{Log}\,(p/1\text{-}p) = ß_0 + ß_1 x$$

$$p = \text{probability of persistent LLV} = 1$$

$$x = \text{exposure variable}$$

For the analyses including hair ARV concentrations, we used only the subset of participants with at least 1 hair measurement available. Hair levels were first summarized by anchor drug (median, interquartile range) for ATV, LPV, NVP, EFV, DRV, and RAL, and compared across VL outcome categories using Kruskal Wallis tests. Next, the proportion of women with a nadir hair concentration in each quartile was summarized by

VL outcome category and compared using a chi-square test. The association between the other potential exposure variables described in Aim 1 (age, race/ethnicity, pre-cART VL, pre-cART CD4 cell count, self-reported medication adherence in the last 6 months, cART regimen type, and year of cART initiation) and the primary exposure of interest (nadir hair quartile) was evaluated using one-way analysis of variance. Bivariate analysis was performed between the nadir hair quartile and the outcome of interest (persistent LLV) using a logistic regression model including dummy variables for each quartile nadir hair concentration, and used this model to estimate unadjusted odds ratios for each nadir quartile hair concentration:

$$\text{Model: Log } (p/1\text{-}p) = ß_0 + ß_1x_1 + ß_2x_2 + ß_3x_3$$

$$p = \text{probability of persistent LLV} = 1$$

$$x_1 = \text{Quartile 2}, x_2 = \text{Quartile 3}, x_3 = \text{Quartile 4}$$

Finally, we created two multivariable logistic regression models including a subset of these exposure variables. The first model did not include hair ARV concentration as an exposure variable because it was designed to evaluate the association between the other potential exposure variables and persistent LLV using the full dataset and estimate adjusted odds ratios for each potential exposure. The second model included only the subset of women with at least 1 hair ARV measurement and included nadir hair ARV concentration as the primary exposure of interest. The exposure variables selected for inclusion in both models were based on the causal diagram (Figure 1) and whether relationships were noted between the primary exposure of interest (hair ARV concentration) or outcome (persistent LLV) in the bivariate analyses. Self-reported adherence was excluded from the second model because hair ARV concentration (the

exposure of interest) is part of the causal pathway between adherence and the outcome of interest. Dummy variables were created for all categorical exposure variables that had more than 2 levels.

$$\text{Example Model 1: } \text{Log } (p/1\text{-}p) = ß_0 + ß_1x_1 + ß_2x_2 + \ldots$$

p = probability of persistent LLV = 1

x_1 = exposure 1, x_2 = exposure variable 2, etc.

$$\text{Example Model 2: } \text{Log } (p/1\text{-}p) = ß_0 + ß_1x_1 + ß_2x_2 + ß_3x_3 + ß_4x_4 + ß_5x_5 + \ldots$$

p = probability of persistent LLV = 1

x_1 = Quartile 2, x_2 = Quartile 3, x_3 = Quartile 4, x_4 = exposure 1, x_5 = exposure variable 2, etc.

Analyses were performed using SAS software, version 9.3 (SAS Institute, Cary, NC).

RESULTS

Study population and VL outcomes:

Of 2,213 WIHS participants contributing 33,676 person-visits of follow-up from 2003-2012, 1,320 WIHS participants met inclusion criteria for this analysis and contributed 11,308 person-visits of follow-up (median 6.5 visits per participant, interquartile range 3 – 15 visits).

Among the included participants, 28.8% (95% CI 26.3–31.2%) had virologic failure, 14.4% (95% CI 12.4–16.3%) had persistent LLV, 25.5% (95% CI 23.1–27.8%) had intermittent LLV, and 31.4% (28.9–33.9%) had sustained viral suppression (Table 1). Women with virologic failure and sustained viral suppression contributed less person-visits of follow-up per participant than women with persistent or intermittent LLV. Women with persistent LLV had a significantly higher median maximum VL (320 vs. 122, $p<0.0001$) and higher median number of person-visits with detectable virus (3 vs. 1, $p<0.0001$) compared to women with intermittent LLV (Figure 2).

Participants in this analysis had a mean age of 44 years, and 54% were African-American, 31% were Hispanic, 14% were white, and 3% reported another race/ ethnicity. Women had a mean pre-treatment VL of 4.11 $\log_{10}$ copies/mL and pre-treatment CD4 cell count of 331 cells/mm^3. Overall, 83% reported ≥95% adherence in the preceding 6 months before the first visit in the analytic follow-up period. The majority of women reported receiving an NNRTI-containing regimen (55%), while 36% received a PI-containing regimen, 7% received an NRTI only regimen, and 2% received an integrase inhibitor-containing regimen; 62% began cART before 2000.

Associations between exposure variables and VL outcomes

Among baseline demographic and clinical characteristics evaluated as potential exposure variables, there were no significant differences observed for age, race/ ethnicity, pre-cART VL, or pre-cART CD4 cell count by VL outcome category in bivariate analysis (Table 2). Self-reported adherence, cART regimen type, and year of cART initiation were significantly associated with VL outcome category in bivariate analysis; women with virologic failure reported lower self-reported adherence, more frequently received PI-based cART, and started cART earlier (Table 2). When VL outcomes were dichotomized to compare participants with the outcome of interest, persistent LLV, versus participants with intermittent LLV or viral suppression, only receipt of PI-based (vs. NNRTI-based) cART was significantly associated with persistent LLV in bivariate analysis (OR 1.58, 95%CI 1.10–2.27, Table 3). In a multivariable logistic regression model including self-reported adherence, cART regimen type, and year of cART initiation, receipt of PI-based (vs. NNRTI-based) cART was significantly associated with persistent LLV (adjusted OR 1.55, 95% CI 1.08–2.24, Table 4).

Associations between hair ARV concentrations, exposure variables, and VL outcomes

There were 797 participants included in the hair ARV concentration analyses and they had 3,125 person-visits with hair concentration data available. The subset of participants with hair ARV concentration data available did not significantly differ from the overall group in any of the demographic or clinical characteristics, except for cART regimen type. No participants included in the hair analysis were receiving NRTI only regimens (since these drugs were not measured in hair). Excluding participants who received NRTI only regimens, participants included in the hair analysis were more likely

to receive PI-containing cART than the overall group of participants meeting inclusion criteria (63% vs. 54%, p=0.002).

Descriptive statistics of hair ARV concentrations across all person-visits by drug and VL outcome category are shown in Table 5. Median hair concentrations of ATV, LPV, DRV, EFV, and NVP were significantly associated with VL outcome category (all $p<0.05$), with lower median concentrations noted for all 5 drugs in the virologic failure group. When VL outcomes were dichotomized to compare participants with persistent LLV versus those with intermittent LLV or viral suppression, only hair concentrations of DRV, EFV, and NVP were associated with persistent LLV (p=0.009, <0.0001, and 0.02, respectively). Women with persistent LLV had higher levels of EFV but lower levels of DRV and NVP compared to women with intermittent LLV or viral suppression.

When the quartile of the nadir hair drug concentration across the follow-up period was determined for each participant and pooled for analysis, a significant association was noted between nadir hair ARV concentration quartile and VL outcome category ($p<0.0001$), with participants with virologic failure more likely to have nadir drug concentrations in the lowest quartile (Table 6). When VL outcomes were dichotomized to compare participants with persistent LLV versus those with intermittent LLV or viral suppression, no significant association between nadir hair ARV concentration quartile and persistent LLV was noted in bivariate analysis (Table 7).

Bivariate analysis was conducted to assess the association between nadir hair concentration quartile and the other exposure variables of interest (age, race/ethnicity, pre-cART VL and CD4 count, self-reported adherence, cART regimen type, and year of cART initiation. Self-reported adherence, year of cART initiation, and cART regimen

type were significantly associated with nadir hair concentration quartile in bivariate analysis (one-way analysis of variance $p<0.05$). Because these exposures were associated with both nadir hair concentration quartile (the exposure of interest) and persistent LLV (the outcome of interest), they were included with nadir hair concentration quartile in a multivariable logistic regression model to estimate the association between exposures and persistent LLV. Self-reported adherence was not included, however, based on the causal diagram (Figure 1). In this multivariable logistic regression model, nadir ARV concentration quartile was not associated with persistent LLV when controlling for cART regimen type and year of ART initiation.

DISCUSSION/CONCLUSIONS

In this large, observational cohort of HIV-infected women in the United States on cART, persistent LLV and virologic failure were common. Notably, as demonstrated by previous studies, self-reported adherence, PI-based cART, earlier cART initiation, and hair ARV concentration were associated with virologic outcome. These associations were driven mostly by the group with virologic failure. Unlike virologic failure, persistent LLV prevalence was not impacted by self-reported adherence, year of cART initiation, or hair ARV concentration.

Nearly one-third of HIV-infected women in this cohort experienced virologic failure, higher than historically reported from predominantly male cohorts, despite high levels of self-reported adherence to cART. Additionally, virologic failure occurred early, with half of women failing by the third biannual study visit after achieving VL <1000 copies/mL. This high prevalence of virologic failure may be due to the fact that the WIHS cohort enrolled HIV-infected women during the 1990s-2000s, during which most women were presumably heavily ARV experienced with likely accumulated drug resistance, likely increasing their risk for virologic failure. However, even among women who began cART during or after 2000, over 20% experienced virologic failure, suggesting an ongoing need to improve treatment outcomes in this group.

Previous studies have suggested suboptimal responses to cART among HIV infected women. WIHS participants are largely African-American or Hispanic, up to half live below the poverty line, and one-third are uninsured (36, 37). In one recent study demonstrating higher rates of treatment discontinuation among HIV-infected Swiss women compared with men, gender disparities in viral suppression were mostly

eliminated when the analysis was adjusted for sociodemographic factors (41). Pharmacokinetic parameters may also impact women's response to cART. Women have exhibited decreased tolerability of NRTIs (42), more severe side effects with PIs (43), and greater drug concentrations and adverse events with the NNRTI nevirapine (44). The high prevalence of virologic failure of the WIHS participants in our sample may therefore be multifactorial, due to earlier cART initiation, sociodemographic factors, decreased ARV tolerability, and unfavorable pharmacokinetics. Future studies of sex differences in persistent LLV and other virologic outcomes could be performed by comparing data from this analysis to data obtained from predominantly male cohorts such as the Multicenter AIDS Cohort Study (MACS).

Notably, persistent LLV occurred in this cohort with similar frequency as previously reported from predominantly male cohorts (6-10). Women who initiated cART later (2000 onward) had a lower prevalence of virologic failure, but a similar prevalence of persistent LLV as those who initiated cART earlier. Despite contemporary cART regimens being more tolerable, persistent LLV prevalence was not lower among women who initiated cART during more recent years. This corresponds with the lack of relationship between drug exposure (measured by hair drug concentrations) and persistent LLV in our study.

The lack of association between persistent LLV and hair ARV concentration noted in our analysis may be due to several factors. First, our data may reflect a true lack of association between persistent LLV and ARV exposure. The etiology of persistent LLV is still controversial, but may be due to factors that are independent of ARV concentration. Up to 80% of individuals on cART who have a VL below the limit of

detection of commercial assays have been shown to demonstrate stable residual viremia in the single copy-number range (24). Studies of viral decay dynamics suggest that low-level viremia may originate from a long-lived compartment of latently infected cells that periodically release virions even in the presence of ART (24, 45). Additional studies using the WIHS cohort could be performed to determine if such extremely LLV is associated with drug concentration using these ultrasensitive viral load assays. Another possibility consistent with our findings is that persistent viremia instead derives from replicating virus in tissues with insufficient ARV concentration or poor ARV penetration (46), which may not be reflected in hair sampling.

Our findings suggest that adherence and exposure to ARV drugs is adequate among patients with persistent LLV or at least similar to the exposure occurring in patients with viral suppression. Rather than increasing adherence counseling, our analysis suggests that clinicians facing the challenge of persistent LLV may need to resort to more aggressive interventions to prevent impending drug resistance and virologic failure. Recent research has shown that ART modification can achieve viral suppression in patients with persistent LLV (9). Interestingly, our analysis demonstrated that women receiving PI-containing ART had increased odds of persistent LLV compared with women receiving NNRTI-containing regimens. PI-based ART regimens have been shown to be inferior to NNRTI-based regimens in suppressing VL in some previous studies (9, 19). Further investigation is needed to determine if specific regimen changes can eliminate the poor virologic outcomes that are associated with persistent LLV.

A limitation in this analysis is that there were several potential sources for misclassification or selection bias. Antiretroviral drug history was obtained by self-report,

so some women reporting cART may not actually have been taking cART during the follow-up period and may have been inadvertently included in the analysis. WIHS mitigates this bias by encouraging women to bring pill bottles to each study visit. Additionally, viremia episodes that occurred between biannual study visits may have been missed, resulting in misclassification of some women into the viral suppression outcome category. The number of person-visits included in follow-up was lower for women with viral suppression than for women with intermittent or persistent LLV, raising the possibility that some women categorized as having viral suppression could fall in other outcome categories if their follow-up period were longer. However, when we included duration of follow-up or duration on cART in multivariable models, our findings did not substantially change. Furthermore, the lack of published ranges for DRV and RAL concentrations in hair may have resulted in use of concentrations outside of the true validated ranges in analysis and thus resulted in misclassification of hair quartiles. Finally, the LLD of VL assays slightly varied over the course of the study, so VL results with a higher LLD could have actually been misclassified as undetectable rather than LLV if a lower LLD was used. However, this is not likely to have occurred frequently since nearly all VL measurements used had a LLD < 80 copies/mL.

Our findings should be interpreted in light of certain additional limitations. First, the analysis included many women who started cART before 2000 and therefore may not be generalizable to current populations of HIV-infected women. Only small numbers of participants had certain exposures (for example, integrase inhibitor-based cART and young age), limiting our ability to assess the effects of these exposures. These limitations can be reduced by conducting additional analyses once more WIHS participants are

enrolled from newer WIHS sites. Second, unmeasured variables could contribute to confounding. We used hair concentrations as our primary exposure of interest, however, because it is the most direct available measure of drug exposure (compared with other adherence measures). Third, we used exposure variables at the start of the follow-up period and did not account for variation over time, such as cART regimen change or changes in self-reported adherence. Further analyses will incorporate these time-varying variables. Finally, we used the nadir hair concentration for each participant in this analysis, whereas measurement of hair concentration occurring before the start of the viremia episode may be most relevant. However, since visits occured only biannually and hair concentration measures drug exposure over weeks to months, use of the value obtained in the study visit before the viremia episode might still not reflect the concentration at the time of the viremia episode. We intentionally selected the nadir concentration so that each woman's "worst case scenario" in terms of drug exposure could be reflected in the analysis (15).

The success of cART has provided hope that a cure for HIV is in sight and resulted in substantial scientific interest in this area (12). Thus far, ART has not achieved such a cure because it cannot completely eradicate HIV from infected individuals. Importantly, our study suggests that increasing antiretroviral drug doses or systemic levels alone may not be enough for HIV eradication or cure. The strengths of our analysis include evaluation of HIV-infected women, a group in which cure research has been limited, standardized longitudinal collection of clinical and laboratory measurements, and the ability to use hair ARV concentrations as a more robust measure of drug exposure than adherence measures used in previous analyses.

In conclusion, persistent LLV and virologic failure were common among this observational cohort of HIV-infected women on cART. Despite the availability of more tolerable and practical therapies in the contemporary age of HIV treatment, the prevalence of persistent LLV was not impacted by year of cART initiation but may be impacted by use of PI-based cART regimens. Finally, concentrations of commonly used anchor drugs in hair samples, while associated with virologic failure, were not associated with persistent LLV. Future research is needed to elucidate the etiology of persistent LLV, especially as it related to ARV exposure, suboptimal adherence, and viral resistance, in order to improve treatment outcomes for HIV-infected women.

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TABLES AND FIGURES

Figure 1. Proposed causal diagram describing the hypothesized relationship between the exposure of interest (hair antiretroviral [ARV] drug concentration), highlighted in blue, and the outcome of interest (persistent low-level viremia [LLV]), highlighted in green. Additional covariates evaluated in this analysis are highlighted in red.

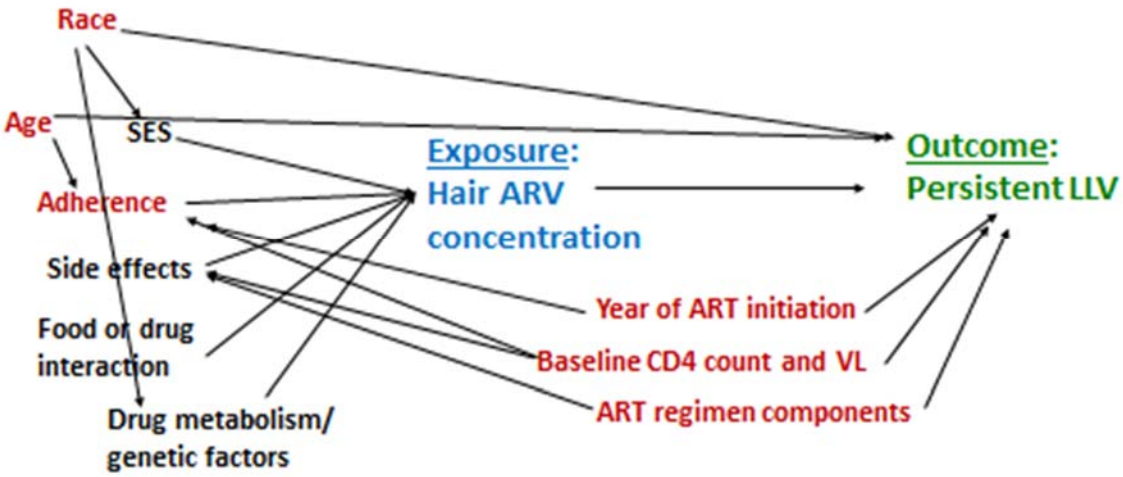
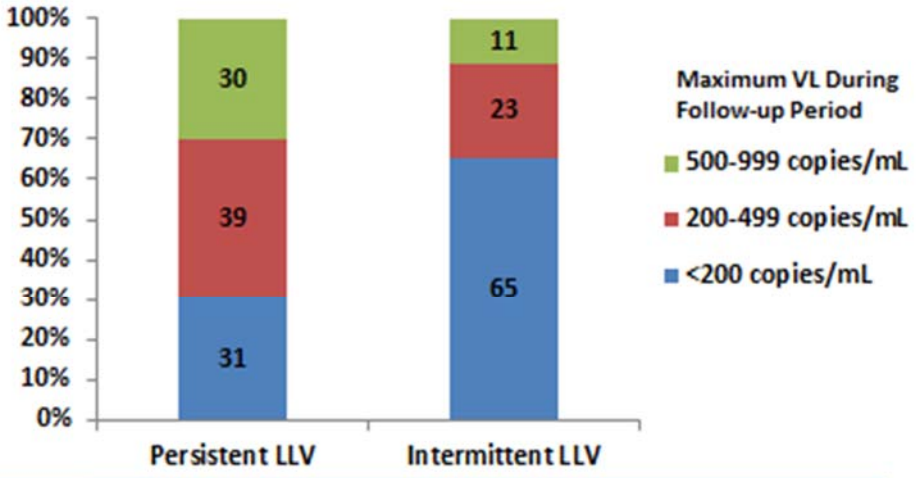


Table 1. Summary of prevalence estimates and follow-up data by viral load (VL) outcome category

VL Outcome Category	Number of women (%, 95% CI*) N = 1296	Number of person-visits (%) N=11283	Person-visits follow-up per participant, Median (IQR)
Virologic failure	373 (28.8, 26.3–31.2)	1802 (16.0)	3 (2–7)
Persistent LLV	186 (14.4, 12.4–16.3)	2210 (19.6)	13 (6–18)
Intermittent LLV	330 (25.5, 23.1–27.8)	3764 (33.4)	12 (5–18)
Viral suppression	407 (31.4, 28.9–33.9)	3507 (31.1)	6 (3–16)

Abbreviations: CI, confidence interval; IQR, interquartile range
*CI = Confidence interval, estimated as $\hat{p} \pm Z_{\alpha/2} \sqrt{\hat{p}(1 - \hat{p})/n}$

Figure 2. Maximum plasma viral load (VL) for women with persistent versus intermittent low-level viremia (LLV)



Maximum VL (Median, IQR copies/mL)	320 (143–546)	122 (62–263)	Wilcoxan Rank Sum p<0.0001
Number of person-visits with LLV (Median, IQR)	3 (2–5) (Maximum 17)	1 (1–2) (Maximum 5)	Wilcoxan Rank Sum p<0.0001

Table 2. Frequency of exposure variables by viral load outcome (table shows number, %, unless otherwise noted).

Variable	Virologic failure N=373	Persistent LLV N=186	Intermittent LLV N=330	Viral suppression N=407	p-value*
Age, mean years (SD)	43 (9)	44 (10)	43 (8)	44 (9)	0.50†
Race/ethnicity					0.22
White	46 (12)	26 (14)	43 (13)	71 (17)	
African-American	214 (57)	93 (51)	190 (58)	199 (49)	
Hispanic	104 (28)	56 (30)	86 (26)	121 (30)	
Other	9 (2)	9 (5)	10 (3)	15 (4)	
Pre-cART viral load, mean log10 c/mL (SD)	4.22 (0.97)	4.19 (0.98)	4.12 (1.02)	4.03 (1.03)	0.51†
Pre-cART CD4 cell count, mean cells/mm^3 (SD)	339 (208)	292 (174)	328 (205)	345 (263)	0.46†
Self-reported adherence (last 6 mo)					**0.002**
≥95%	289 (77)	162 (87)	269 (82)	349 (86)	
75-94%	63 (17)	19 (10)	54 (16)	48 (12)	
0-74%	21 (6)	5 (3)	5 (2)	9 (2)	
cART regimen type					**<0.0001**
NNRTI	89 (26)	56 (33)	122 (39)	166 (44)	
PI	224 (66)	100 (60)	157 (51)	169 (45)	
Integrase inhibitor	3 (1)	2 (1)	6 (2)	10 (3)	
NRTI only	22 (7)	10 (6)	24 (8)	29 (8)	
Year of cART initiation					**<0.0001**
1995-1999	266 (72)	115 (62)	205 (62)	220 (54)	
2000-2003	71 (19)	30 (16)	66 (20)	82 (20)	
2004-2008	29 (8)	24 (13)	31 (9)	61 (15)	
After 2008	6 (2)	17 (9)	28 (8)	43 (11)	

Abbreviations: LLV, low-level viremia; SD, standard deviation; c/ml, copies per milliliter; cART, combination antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor

* Two-tailed chi-square *p*-value except where noted, α=0.05, significant *p*-values marked in bold

† One-way analysis of variance *p*-value

Table 3. Bivariate association between exposure variables and persistent LLV (vs. viral suppression or intermittent LLV)

Variable	Persistent LLV N=186 n (%)	Virologic suppression or intermittent LLV N=737 n (%)	OR (95% CI)*
Age			
< 30 years	11 (6)	32 (4)	Reference
30-39 years	48 (26)	223 (30)	0.63 (0.30–1.33)
40-49 years	76 (41)	324 (44)	0.68 (0.33–1.41)
≥ 50 years	51 (27)	157 (21)	0.95 (0.44–2.01)
African-American race	91 (49)	346 (47)	0.91 (0.66–1.26)
Pre-cART VL >100,000 c/mL	14 (24)	42 (17)	1.59 (0.80–3.16)
Pre-cART CD4 count <200 cells/mm^3	20 (33)	76 (29)	1.22 (0.67–2.22)
Self-reported adherence <95%	24 (13)	116 (16)	0.79 (0.49–1.27)
cART regimen type			
NNRTI	56 (33)	288 (42)	Reference
PI	100 (60)	326 (48)	1.58 (1.10–2.27)
Integrase inhibitor	2 (1)	16 (2)	0.64 (0.14–2.87)
NRTI only	10 (6)	53 (8)	0.97 (0.47–2.02)
Year of first cART 1995-1999 (vs. after 2000)	115 (62)	425 (58)	0.84 (0.61–1.17)

Abbreviations: LLV, low-level viremia; VL, viral load; c/ml, copies per milliliter; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; cART, combination antiretroviral therapy

* Unadjusted Odds Ratio (OR) and 95% confidence interval (CI) from logistic regression model $\log (p/1-p) = ß_0 + ß_1x$; p = probability of persistent LLV = 1

Table 4. Multivariable logistic regression model* of predictors of persistent low-level viremia outcome, not including hair antiretroviral drug concentration

Variable	Parameter estimate	Standard error	Wald χ^2	*p*-value	OR (95% CI)†
Self-reported adherence					
≥95%	--	--	--	--	Reference
75-94%	-0.26	0.28	0.87	0.351	0.77 (0.45, 1.33)
0-74%	0.33	0.59	0.30	0.582	1.39 (0.43, 4.45)
cART regimen type					
NNRTI	--	--	--	--	Reference
PI	0.44	0.19	5.55	0.018	1.55 (1.08, 2.24)
Integrase inhibitor	-0.40	0.77	0.27	0.601	0.67 (0.15, 3.01)
NRTI only	-0.03	0.38	0.00	0.946	0.98 (0.47, 2.04)
cART initiation 2000 onward (vs. before)	0.08	0.18	0.22	0.637	1.09 (0.77, 1.54)

Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; cART, combination antiretroviral therapy

* Intercept (standard error): -1.65 (0.18)

† Odds Ratio (OR) and 95% Confidence Interval (CI), including other listed exposure variables in the model

Table 5. Descriptive statistics of hair antiretroviral drug concentrations within validated ranges across all person-visits by viral load outcome category

Median drug concentration, ng/mL (IQR)*	**Overall (N=797)**	**Virologic failure (N=221)**	**Persistent LLV (N=122)**	**Intermittent LLV (N=213)**	**Viral suppression (N=241)**
ATV (n=314, 1085 pv)†	3.13 (1.83, 5.24)	1.89 (0.86, 3.80)	2.06 (0.91, 4.22)	2.27 (1.36, 4.39)	2.72 (1.64, 4.22)
LPV (n=129, 575 pv) †	5.20 (2.64, 7.28)	1.45 (0.73, 2.81)	3.69 (3.07, 4.42)	4.24 (3.08, 6.50)	5.41 (3.04, 8.12)
DRV (n=57, 169 pv) †‡	6.17 (3.90, 9.61)	3.88 (2.03, 4.13)	5.51 (2.76, 7.15)	5.29 (2.87, 8.79)	5.00 (2.57, 9.20)
EFV (n=164, 689 pv)†‡	4.55 (2.64, 7.28)	2.79 (1.04, 5.18)	4.25 (2.95, 8.48)	3.16 (1.91, 5.11)	3.21 (1.82, 5.08)
NVP (n=130, 579 pv)†‡	32.13 (18.94, 48.79)	15.04 (5.60, 29.68)	21.39 (3.71, 35.03)	17.17 (7.32, 43.04)	30.50 (10.09, 43.57)
RAL (n=4, 77 pv)	0.67 (0.28, 1.43)	0.22 (0.22, 0.22)	Data not available	1.57 (1.57, 1.57)	1.96 (1.42, 2.51)

Abbreviations: LLV, low-level viremia; pv, person-visits with hair data available; ATV, atazanavir; LPV, lopinavir; DRV, darunavir; EFV, efavirenz; NVP, nevirapine; RAL, raltegravir

*Hair antiretroviral drug concentrations are those falling within validated ranges for ATV, LPV, EFV, and NVP, and were measured at any study visit in the analytic follow-up period.

† Kruskal Wallis *p*-value <0.05 comparing values for women across VL outcome groups

‡ Kruskal Wallis *p*-value <0.05 comparing women with persistent LLV to women with intermittent LLV or viral suppression.

Table 6. Frequency of nadir hair drug concentration quartile during follow-up period by viral load outcome

Quartile of nadir hair ARV concentration	Virologic failure N=221 n (%)	Persistent LLV N=122 n (%)	Intermittent LLV N=213 n (%)	Viral suppression N=241 n (%)	p-value*
Quartile 1 – Lowest	127 (57)	44 (36)	85 (40)	81 (34)	
Quartile 2	45 (20)	39 (32)	62 (29)	60 (25)	
Quartile 3	28 (13)	21 (17)	28 (13)	58 (24)	**<0.0001**
Quartile 4 – Highest	21 (10)	18 (15)	38 (18)	42 (17)	

Abbreviations: ARV, antiretroviral; LLV, low-level viremia

* Two-tailed chi-square *p*-value, α=0.05, significant *p*-value marked in bold

Table 7. Bivariate association between nadir hair drug concentration quartile and persistent LLV (vs. viral suppression or intermittent LLV)

Quartile of nadir hair ARV concentration	**Persistent LLV N=122 n(%)**	**Virologic suppression or intermittent LLV N=454 n(%)**	**OR (95% CI)***
Quartile 1 – Lowest	44 (36)	166 (37)	Reference
Quartile 2	39 (32)	122 (27)	1.21 (0.74-1.97)
Quartile 3	21 (17)	86 (19)	0.92 (0.52-1.65)
Quartile 4 – Highest	18 (15)	80 (18)	0.85 (0.46-1.56)

Abbreviations: ARV, antiretroviral; LLV, low-level viremia

* Unadjusted Odds Ratio (OR) and 95% confidence interval (CI) from logistic regression model

Table 8. Multivariable logistic regression model* of predictors of persistent low-level outcome, including hair antiretroviral drug concentration

Variable	Parameter estimate	Standard error	Wald χ^2	*p*-value	OR (95% CI)†
Nadir hair drug concentration					
Quartile 1	--	--	--	--	Reference
Quartile 2	-0.001	0.27	0.00	0.997	0.99 (0.59, 1.70)
Quartile 3	-0.20	0.33	0.39	0.533	0.82 (0.43, 1.55)
Quartile 4	-0.23	0.34	0.48	0.490	0.79 (0.41, 1.53)
cART regimen type					
NNRTI	--	--	--	--	Reference
PI	0.33	0.23	1.97	0.161	1.39 (0.88, 2.18)
Integrase inhibitor	-11.98	686.40	0.00	0.986	<.001 (<.001, >999.99)
cART initiation 2000 onward (vs. before)	0.05	0.23	0.05	0.822	1.05 (0.67, 1.64)

Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; cART, combination antiretroviral therapy

* Intercept (standard error): -1.55 (0.24)

† Odds Ratio (OR) and 95% Confidence Interval (CI), including other listed exposure variables in the model