

ORIGINAL RESEARCH

Persistent low-level viraemia and virological failure in HIV-1-infected patients treated with highly active antiretroviral therapy

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Objective

To assess the prognostic significance of persistent low-level viraemia (PLV, defined as persistent plasma viral loads of 51–1000 HIV-1 RNA copies/mL for at least 3 months) in patients who had achieved viral suppression on antiretroviral therapy (ART).

Methods

A retrospective cohort of HIV-infected patients who received ART, were followed-up for ≥ 12 months, made regular visits to the clinic during which blood tests were performed for an ultrasensitive HIV RNA assay every 3 months, and achieved viral loads <50 copies/mL were evaluated. Virological failure was defined as two consecutive viral load measurements >1000 copies/mL.

Results

Of 362 patients, 78 (27.5%) experienced PLV. The demographics of patients with and without PLV were similar. PLV occurred at a mean ($\pm$ standard deviation) of 22.6 ± 16.9 months after ART initiation and lasted for 6.4 ± 3.4 months. During a median follow-up of 29.5 months, patients with PLV had a higher rate of virological failure (39.7% vs 9.2%; $P<0.001$). The median time to failure was 68.4 months [95% confidence interval (CI) 37.0–99.7] for patients with PLV and >72 months for patients without PLV (log rank test, $P<0.001$). By Cox regression, patients with PLV had a greater risk of virological failure [hazard ratio (HR) 3.8; 95% CI 2.2–6.4; $P<0.001$]. Among patients with PLV, a PLV of >400 copies/mL (HR 3.3; 95% CI 1.5–7.1; $P=0.003$) and a history of ART (HR 2.4; 95% CI 1.0–5.7; $P=0.042$) predicted virological failure.

Conclusions

PLV is associated with virological failure. Patients with a PLV >400 copies/mL and a history of ART experience are more likely to experience virological failure. Patients with PLV should be considered for treatment optimization and interventional studies.

Keywords: antiretroviral therapy, HIV RNA, viraemia, virological failure

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Introduction

Since the introduction of highly active antiretroviral therapy (HAART), there has been a dramatic decrease in HIV-related mortality. The goal of HAART in HIV-infected patients is to reduce plasma HIV viral load (HIV RNA) to undetectable levels and to increase the CD4 cell count. Achievement of this goal reduces the rate of disease progression and death. However, some patients experience isolated episodes of transiently detectable HIV RNA or viral rebound. The causes of viral rebound are still unclear. Rates of viral rebound of 25–53% have been reported among patients on HAART who have achieved undetectable HIV RNA [1–6].

While in some studies intermittent viraemia has not been associated with virological failure [7–9], viral rebound has been associated with greater risk of virological failure in

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others [2,10–12]. Work by Raboud *et al.* demonstrated that consecutive rebounds in plasma viral load are associated with virological failure at 52 weeks [6]. A study by Karlsson *et al.* also showed that persistent low-level viraemia significantly increased the risk of subsequent virological failure [13]. However, both studies had small numbers of patients who actually received potent antiretroviral therapy (ART). Raboud *et al.* included 27 patients who were on triple therapy and Karlsson *et al.* enrolled 46 patients on HAART. Some patients with persistent low-level viraemia in both studies did not have virological failure after follow-up for 12–36 months.

The aim of this study was to investigate the prognostic significance of persistent low-level viraemia and to determine risk factors that predict subsequent virological failure in patients with persistent low-level viraemia.

Methods

A retrospective cohort study was performed on HIV-infected patients who received HAART between January 1999 and December 2003 at a university hospital. The inclusion criteria included patients who: (1) received HAART and were followed up for at least 12 months; (2) achieved undetectable HIV RNA (<50 HIV-1 RNA copies/mL) within 6 months of HAART; and (3) made regular visits to the clinic during which blood tests were performed for HIV RNA every 2–3 months. Each eligible patient was followed up until the end of the study (31 December 2004) or until HAART was discontinued. The study was approved by the Washington University Human Studies Committee.

Data collected included demographics, history of antiretroviral therapy (ART), HAART regimen, CD4 cell count and HIV RNA at every visit, and HIV genotype. All tests for CD4, HIV RNA, and HIV genotype were ordered at the discretion of the health care provider in the infectious disease clinic. Plasma HIV RNA was determined using the UltraSensitive Amplicor HIV-1 Monitor version 1.5 (v1.5) kits (Roche Diagnostics, Indianapolis, IN, USA). HIV genotypes were sequenced by fluorescent dideoxy chain termination (Perkin-Elmer, Wellesley, MA, USA or Applied Biosystems, ABI, Foster City, CA, USA). Sequencing results were reported as amino acid changes at positions in the HIV protease and reverse transcriptase genes. The patients' genotype results were compared with reported mutations published in the *Drug Resistance Mutations in HIV-1*, updated in April 2005 by the International AIDS Society (IAS)-USA [14].

Persistent low-level viraemia was defined as persistent plasma HIV RNA levels in the range of 51–1000 copies/mL for at least 3 months and on at least two consecutive clinic visits. Virological failure was defined as two consecutive plasma HIV RNA levels >1000 copies/mL.

The collected data were analysed using the SPSS software package version 12.0 (SPSS, Chicago, IL, USA). Patients were categorized into two groups: with and without persistent low-level viraemia. Univariate analysis was performed to compare baseline characteristics between the two groups. Categorical data were compared using the chi square test or Fisher's exact test where appropriate. Continuous data were compared by two-sample Student's *t*-test for equal variances or the Wilcoxon rank-sum test for variables that were not normally distributed. The distributions of probability of virological failure were estimated using the Kaplan–Meier method and comparisons were made using the log rank test. Patients were classified at time zero when their HAART regimen was initiated. Patients were censored at the end of the study (31 December 2004), at discontinuation of HAART, or at loss to follow-up.

A Cox proportional hazards model was used to evaluate the risk factors for virological failure. Models were fitted for time to virological failure. The time to failure was determined by the first HIV RNA level >1000 copies/mL. The effect estimate was recorded as the hazard ratio (HR). Another Cox proportional hazards model was used to evaluate the risk factors for virological failure in patients with persistent low-level viraemia. For all statistical tests, a *P*-value of less than 0.05 was considered statistically significant.

Results

There were 362 patients in total, with a mean age of 39.2 ± 9.9 years, of whom 69.9% were male. The study population included 188 African-American patients (51.9%), 168 Caucasian patients (46.4%), five Hispanic patients (1.4%) and one Asian patient (0.3%). Half of the patients had a history of ART experience before initiation of HAART in this study. There were 233 (64.4%) patients who received nonnucleoside reverse transcriptase inhibitor (NNRTI)-based HAART regimens; the other 129 patients (35.6%) received protease inhibitor (PI)-based HAART regimens. Baseline mean CD4 cell count and median (range) HIV RNA at HAART initiation were 316 ± 263 cells/μL and 3.9 (1.6–6.2) log copies/mL, respectively.

Seventy-eight patients (27.5%) developed persistent low-level viraemia, which occurred at a mean of 22.6 ± 16.9 months after initiation of HAART and 18.8 ± 11.2 months after they had achieved undetectable HIV RNA. In patients who developed persistent low-level viraemia, mean duration of persistent low-level viraemia was 6.4 ± 3.4 months, equivalent to between two and five consecutive clinic

Table 1 Baseline characteristics of patients with and without persistent low-level viraemia

Characteristic	Patients with persistent low-level viraemia ($n = 78$)	Patients without persistent low-level viraemia ($n = 284$)	*P*-value
Age (years) (mean ± SD)	39.7 ± 9.6	39.0 ± 9.9	0.582
Gender [*n* (%)]			0.127
Male	60 (76.9)	193 (68.0)	
Female	18 (23.1)	91 (32.0)	
Race [*n* (%)]			0.449
White	33 (42.3)	135 (47.5)	
African-American	44 (56.4)	144 (50.7)	
Hispanic	1 (1.3)	4 (1.4)	
Asian	0	1 (0.4)	
History of ART [*n* (%)]			1.000
Naive	39 (50)	142 (50)	
Experienced	39 (50)	142 (50)	
Mean baseline CD4 (cells/μL)	294.6 ± 233.5	322.4 ± 281.9	0.424
Mean baseline $\log_{10}$ HIV RNA (copies/mL)	4.0 ± 1.4	3.8 ± 1.5	0.410
HAART regimen [*n* (%)]			0.748
NNRTI-based	49 (62.8)	184 (64.8)	
PI-based	29 (37.2)	100 (35.2)	
Follow-up time (months) [median (range)]	32.0 (12.2–78.2)	29.0 (15.0–84.4)	0.379

HAART, highly active antiretroviral therapy; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

visits. The mean HIV RNA level for persistent viraemia was 258 ± 139 copies/mL (range 53–997 copies/mL). Demographics and baseline characteristics, including age, gender, race, history of ART, baseline CD4 cell count, baseline HIV RNA and treatment regimens, did not differ significantly between the 78 patients with persistent low-level viraemia and the 284 patients without persistent low-level viraemia (Table 1). Median time to achieve undetectable HIV RNA (< 50 copies/mL) was 4.3 months in patients with persistent low-level viraemia and 3.6 months in patients without persistent low-level viraemia ($P = 0.278$).

During a median follow-up period of 29.5 months (range 12.2–84.4 months), patients with persistent low-level viraemia had a higher rate of virological failure (39.7% vs 9.2%; $P < 0.001$). CD4 count changes from baseline between the two groups were not different (median 213 cells/μL in patients without persistent low-level viraemia vs 190 cells/μL in patients with persistent low-level viraemia; $P = 0.281$). From Kaplan–Meier analysis (Fig. 1), the median time to failure was 68.4 months [95% confidence interval (CI) 37.0–99.7] for patients with persistent low-level viraemia and > 72 months for patients without persistent low-level viraemia (log rank test; $P < 0.001$). In the Cox proportional hazards model, patients with persistent low-level viraemia had a significantly greater risk of virological failure [hazard ratio (HR) 3.8;

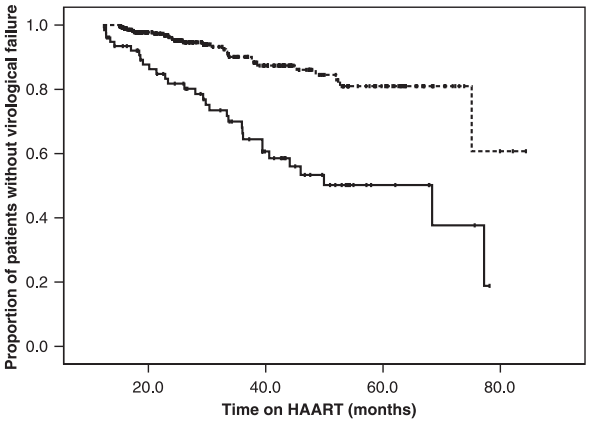

Fig. 1 Kaplan–Meier analysis showing the probability of virological failure in patients with and without persistent low-level viraemia. The solid line represents patients with persistent low-level viraemia, and the dashed line represents those with sustained undetectable HIV RNA. The groups were significantly different by a log rank test ($P < 0.001$). HAART, highly active antiretroviral therapy.

95% CI 2.2–6.4; $P < 0.001$). Other factors included in the models but not predictive of failure were baseline CD4 cell count, baseline HIV RNA, HAART regimens, and history of ART.

The group of 78 patients who had persistent low-level viraemia were studied for risk factors to determine failure in the population. A comparison of the baseline characteristics of 31 patients who had virological failure and 47 patients who did not have virological failure is shown in Table 2. By Cox regression, a persistent viraemia of > 400 copies/mL (HR 3.3; 95% CI 1.5–7.1; $P = 0.003$) and a history of ART (HR 2.4; 95% CI 1.0–5.7; $P = 0.042$) predicted virological failure. Baseline CD4 cell count and duration of persistent low-level viraemia did not predict virological failure (Table 3).

Of a total of 57 patients with virological failure, 51 had been tested for HIV genotype. Among these 51 patients, 15 had this test carried out at the time of persistent low-level viraemia with a median plasma HIV RNA of 417 copies/mL (range 106–954 copies/mL). Ten of these 15 patients (66.7%) were found to have new primary mutations contributing to antiretroviral resistance. The most common primary mutations were M184V (six patients; 40.0%), thymidine analogue mutations (TAMs) (three patients; 20.0%) and K103N (two patients; 13.3%).

Discussion

In clinical practice, we observed that many patients who experienced persistent low-level viraemia, unlike those with intermittent viraemia (blips, defined as an HIV RNA

Table 2 Baseline characteristics of 78 patients with persistent low-level viraemia

Characteristic	Patients with persistent low-level viraemia and virological failure ($n = 31$)	Patients with persistent low-level viraemia but no failure ($n = 47$)	*P*-value
Age (years) (mean ± SD)	40.3 ± 8.7	38.8 ± 10.8	0.503
Gender [*n* (%)]			0.644
Male	8 (25.8)	10 (21.3)	
Female	23 (74.2)	37 (78.7)	
Race [*n* (%)]			0.391
White	11 (35.5)	22 (46.8)	
African American	20 (64.5)	24 (51.1)	
Hispanic	0	1 (2.1)	
History of ART [*n* (%)]			0.818
Naive	15 (48.4)	24 (51.1)	
Experienced	16 (51.6)	23 (48.9)	
Mean baseline CD4 (cells/μL)	297.8 ± 242.6	289.7 ± 223.0	0.883
Mean baseline $\log_{10}$ HIV RNA (copies/mL)	4.1 ± 1.5	3.8 ± 1.4	0.424
HAART regimen [*n* (%)]			0.098
NNRTI-based	16 (51.6)	33 (70.2)	
PI-based	15 (48.4)	14 (29.8)	
Follow-up time (months) [median (range)]	29.2 (12.2–78.2)	33.7 (14.7–75.8)	0.300

HAART, highly active antiretroviral therapy; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Table 3 Cox regression of factors related to virological failure in 78 patients with persistent low-level viraemia

Characteristic	Hazard ratio	95% confidence interval	*P*-value
History of ART experience	2.43	1.03–5.71	0.042
Baseline CD4 < 200 cells/μL	1.25	0.55–2.83	0.594
HIV RNA level of persistent low-level viraemia > 400 copies/mL	3.26	1.50–7.12	0.003
Duration of persistent low-level viraemia > 6 months	1.12	0.54–2.34	0.761

ART, antiretroviral therapy.

measurement of 50–1000 copies/mL that is preceded and followed by another HIV RNA measurement of < 50 copies/mL), had subsequent virological failure. This phenomenon is quite common as we found it occurred in more than 25% of patients who achieved viral suppression in this study. Its prognostic significance needs to be addressed and differentiated from that of HIV viral blips.

This cohort analysis with a median follow up of more than 2 years identified a significantly higher rate of virological failure in patients who experienced persistent low-level viraemia. Persistent low-level viraemia increased the risk of subsequent virological failure approximately fourfold compared with patients with sustained viral suppression. In particular, the occurrence of persistent low-level viraemia at a level of more than 400 copies/mL carried a worse prognosis than lower levels of detectable viral replication.

Our data support current national and international treatment guidelines regarding the management of intermittent vs persistent low-level viraemia [15,16]. While Havlir *et al.* [7] and others [8,9,17] found that intermittent elevations in plasma HIV RNA during treatment were not associated with virological failure, our data and those of others [6,13] have shown that patients with persistent low-level viraemia have a higher risk of virological failure. In addition, two-thirds of HIV genotype tests during persistent low-level viraemia showed new primary mutations contributing to antiretroviral resistance. If possible, modification of therapy in patients experiencing persistent low-level viraemia is indicated. Ongoing viral replication in the presence of suboptimal ART promotes the selection of further drug resistance mutations [18,19].

A potential limitation of our study is that we analysed HIV RNA obtained in clinic visits that were 2–3 months apart. The duration of the detectable viraemia during ART is important in determining its impact on outcome, as transient viraemia does not predict virological failure while persistent low-level viraemia does. The definition of intermittent viraemia or persistent viraemia is dependent on the frequency of sampling. However, the results from our study may be more applicable to real-life clinical practices in which patients are seen and evaluated every 2–3 months, i.e. persistent low-level viraemia in our definition is similar to persistent low-level viraemia seen in clinical practice.

In summary, persistent low-level viraemia for 3 months or more is associated with subsequent virological failure. Among patients experiencing persistent low-level viraemia, those with a level of persistent low-level viraemia > 400 copies/mL and a history of ART experience are more likely to have virological failure. Patients with persistent low-level viraemia should be considered for treatment optimization and intervention studies.

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