Strong and persistent correlation between baseline and follow-up HIV-DNA levels and residual viremia in a population of naïve patients with more than 4 years of effective antiretroviral therapy

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Abstract

In a longitudinal study on 181 naïve patients who responded to therapy (mean follow-up 4 years), high baseline human immunodeficiency virus (HIV)-RNA values correlated with high levels of cellular HIV-DNA at all time points ($p < 0.0001$, p 0.045, p 0.0055, and p 0.0025, respectively) and negatively correlated with undetectable residual viremia (URV; <2.5 copies/mL) at T1, T2, and T3 (p 0.026, p 0.0149, and p 0.0002, respectively). Baseline high HIV-DNA levels predicted the persistence of high values (p 0.0001) and negatively correlated with URV (p 0.0254, p 0.0481, and p 0.0085). These results suggest that baseline viral load, cellular HIV-DNA, and URV were strongly correlated over long-term follow-up of antiretroviral therapy responders.

Keywords: Cellular HIV-DNA, HIV-RNA, naïve patients, primary infection, residual viremia

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Introduction

A few data concerning long-term human immunodeficiency virus (HIV)-DNA monitoring have been reported [1–7] and it is not known how the demonstrated correlations between HIV-DNA levels and the main viro-immunological parameters could change over the course of effective treatment. The purpose of our study was to determine correlations between baseline HIV-RNA, cellular HIV-DNA, undetectable residual viremia (URV, <2.5 copies/mL), CD4 count, and acute or chronic infection at the start of antiretroviral therapy (ART) in naïve patients who responded to ART.

Methods

A longitudinal study was conducted on 181 subjects who achieved and maintained suppression of HIV-RNA levels <50 copies/mL for 4 years (mean): plasma HIV-RNA was determined at least four times per year.

Blood samples were collected at baseline (T0, before the beginning of therapy, nadir) and at the first (T1) and second (T2) follow-up times. For 70 of the 181 patients, a third follow-up time (T3) was included. This study was conducted in accordance with the Helsinki Declaration and local legislation (Ethics Committee of Padova University Hospital, prot. 2606-12P).

Cellular HIV-DNA and residual viremia (RV) were quantified as previously described [1]. A multilevel mixed-effects linear regression with $\log_{10}$ cellular HIV-DNA as dependent variable and conventional descriptive statistics were applied when appropriate.

Most participants were male (76.8%), Italian (80.7%), mean age of 41 years (±11.5), and 30.9% men who have sex with men, 42.5% heterosexual; patients were mainly chronically infected (87.3%) and had HIV subtype B (76.8%). Patients' data were summarized in Table 1.

Results

A higher CD4 count at each follow-up time was related to a greater likelihood of having high levels of CD4+ cells at the subsequent time points (p 0.0001). Fast initial decay of cellular HIV-DNA levels over time was observed during the first 2 years of observation (mean, T1). A slower decay was observed throughout the study.

High values of plasma HIV-RNA at T0 were correlated with high levels of cellular HIV-DNA at all time points (p 0.0001, p

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TABLE 1. Evolution of the number and percentage of CD4+ T cells, cellular HIV-DNA levels, and of number of patients with viremia less of 2.5 copies/mL at T0,T1 and T2 for 181 patients and at T0, T1, T2 and T3 for 70 patients

Laboratory parameters	T0 (181 patients)	T1 (181 patients)	T2 (181 patients)	T0 (70 patients)	T1 (70 patients)	T2 (70 patients)	T3 (70 patients)
CD4+ cells/μL[a]	259 (±174)	563 (±247)	608 (±265)	255 (±176)	560 (±213)	600 (±244)	624 (±241)
CD4+ cells percentage[a]	15.6 (±8.8)	27.6 (±9.8)	29.6 (±9.1)	16.4 (±8.9)	29.4 (±9.2)	30.3 (±8.6)	31.9 (±8.2)
Cellular HIV-DNA copies/ 10^6 PBMCs[a]	2942 (±6491)	256.5 (±443)	141.6 (±156.3)	3121 (±4124)	298 (±557)	167.3 (±190.5)	110 (±112)
Plasma log $_{10}$ HIV-RNA[a]	5.53 (±6.08)	<1.69	<1.69	5.57 (±6.11)	<1.69	<1.69	<1.69
N (%) of patients with HIV-RNA <2.5 copies/mL	0	65 (35.9)	62 (34.2)	0	25 (35.7)	25 (35.7)	27 (38.5)
Time of follow-up (months)[a]		24 ± 12	12 after T1		24 ±12	12 after T1	12 after T2

PBMCs, peripheral blood mononuclear cells.
[a]Mean ± SD.

0.045, p 0.0055, and p 0.0025, respectively) and were negatively correlated with the possibility of reaching URV (p 0.026, p 0.0149, and p 0.0002, respectively). URV at T2 was more frequently achieved by people beginning with <100 000 copies/mL than those with >100 000 plasma HIV-RNA copies/mL (p 0.007).

High levels of cellular HIV-DNA at baseline were correlated with high values of cellular HIV-DNA and were negatively correlated with the achievement of URV at all follow-up time points (Table 2).

At T0 (and after 1, 2, 3, and 4 years), a linear correlation between cellular HIV-DNA and CD4 count was detected; lower levels of cellular HIV-DNA were detected in patients with less than 100 000 copies/mL of plasma HIV-RNA at T0.

Primary infection was associated with a lower likelihood of high levels of cellular HIV-DNA at T1 (p 0.0325), T2 (p 0.0254), and T3 (p 0.0191); conversely, it was associated with a higher probability of presenting high levels of CD4+ cells at baseline (p < 0.0001), T1 (p 0.0006), and T2 (p 0.0011), as well as in achieving URV at T1 (p 0.0398) and T3 (p 0.0086).

These results were confirmed by a multilevel ME linear regression, which demonstrated a negative correlation among DNA values and time, primary infection, and lower RV, and a positive correlation with CD4 count and baseline plasma RNA (Supplementary Table 1).

Discussion

The first relevant result of this study was the evidence that the correlation between the viral load and HIV-DNA at T0 is steadily maintained over time during effective therapy, confirming that HIV-RNA load and cellular HIV-DNA load are the main factors related to the size of the viral reservoir, even long after the beginning of effective therapy.

Second, we showed that high levels of HIV-DNA at all follow-up times were negatively correlated with the achievement of URV, suggesting that the persistence of RV is related to the size of the reservoir.

However, recently, in a cross-sectional study on 243 patients [8], a high pretreatment viral load was the main factor associated with the inability to achieve low levels of HIV-DNA after a long follow-up. In our study, high levels of pretreatment HIV-RNA were correlated with high levels of HIV-DNA at all follow-up times, in a longitudinal perspective.

With respect to the work by Fourati et al. [8], based on multi-experienced patients, our naïve population remained virologically controlled throughout the study period of 4 years.

In a prospective cohort study [9] a low pre-therapy level of HIV-DNA was the only predictor of CD4 cell recovery and the achievement of an undetectable viral load. We confirmed the

TABLE 2. Correlation between achievement of residual plasma viremia <2.5 cps/mL at T1, T2, and T3 and the values of plasma viremia at baseline (prior to initiation of ART) and cellular HIV-1 DNA at baseline, T1, T2, and T3

HIV-1 RNA <2.5 cps/mL		HIV-1 RNA at T0	HIV-1 DNA at T0	HIV-1 DNA at T1	HIV-1 DNA at T2	HIV-1 DNA at T3
At T1	corr	−0.1713	−0.1889	−0.3107	−0.1544	−0.2181
	p	0.026	0.0254	0.0001	0.0438	0.0762
	n	169	140	164	171	67
At T2	corr	−0.1882	−0.1692	−0.1902	−0.244	−0.1465
	p	0.0149	0.0481	0.0154	0.0014	0.2262
	n	167	137	162	168	70
At T3	corr	−0.4921	−0.3919	−0.3595	−0.406	−0.5681
	p	0.0002	0.0085	0.0088	0.0023	0.0000
	n	54	44	52	54	54

ART, antiretroviral therapy; HIV, human immunodeficiency virus.
Coefficient of correlation (corr), p-value (significance), and number of observations (*n*) are reported.

strict correlation between HIV-DNA values and the CD4 count at all follow-up times. We also found an inverse correlation between HIV-DNA levels at baseline and at any subsequent time with the possibility of reaching URV, thus extending the prognostic significance of cellular DNA.

As reported [9], patients with primary infection achieved lower levels of HIV-DNA and higher levels of CD4+ lymphocytes; our study confirms these results on patients without previous failures, adding data on the highest probability of achieving URV at different time points.

Recently, no association was found between HIV-DNA slopes and residual viremia in a longer follow-up, but these valuable data were based on fewer subjects (n = 18), and need further elucidations [10].

The main limitation of our study was the evaluation of HIV-DNA using a PCR-based assay that does not allow the replication-competent and replication-defective forms of the viral genome to be distinguished. Nevertheless, unintegrated HIV-DNA became undetectable during the first year of highly active antiretroviral therapy, and total and integrated HIV-DNA levels were generally equal in well-controlled patients [11]. Our study, encompassing a longitudinal perspective with multiple set points for each patient, suggests that there is a good and long-lasting correlation between plasma viral load, CD4 count, and cellular HIV-DNA before and after the start of ART. The amount of cellular HIV-DNA is predictive of the immune recovery and is closely related to each other with the control of RV over a long-term follow-up, in a cohort of naïve and successfully treated patients. All these correlations are more evident in the population of patients treated for a primary infection.

Transparency declaration

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2014.10.009.

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