

Association between Cellular Human Immunodeficiency Virus DNA Level and Immunological Parameters in Patients with Undetectable Plasma Viremia Level during Highly Active Antiretroviral Therapy

Loredana Sarmati,¹ Saverio Giuseppe Parisi,⁶ Emanuele Nicastrì,² Gabriella d'Ettorre,⁴
Lucia Palmisano,⁵ Mauro Andreotti,⁵ Carolina Andreoni,⁴ Marina Giuliano,⁵
Francesca Gatti,³ Caterina Boldrin,⁶ Giorgio Palù,⁶ Vincenzo Vullo,⁴
Stefano Vella,⁵ and Massimo Andreoni^{1*}

Department of Public Health and Cellular Biology, University of Rome Tor Vergata, Rome,¹ National Institute for Infectious Diseases, IRCCS L. Spallanzani, Rome,² Institute of Immunology and Infectious Diseases, University of Verona, Verona,³ Department of Infectious Diseases University of Rome La Sapienza, Rome,⁴ Istituto Superiore di Sanità, Rome,⁵ and Department of Microbiology, Padua University Hospital, Padua,⁶ Italy

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The association between human immunodeficiency virus (HIV) DNA load and immunologic parameters was investigated in 163 HIV-infected patients with undetectable plasma viremia during highly active antiretroviral therapy (HAART). Patients with HIV DNA below the 25th percentile (133 copies/10⁶ peripheral blood mononuclear cells) had higher pre-HAART ($P = 0.001$) and current ($P = 0.005$) CD4 counts and a prolonged duration of treatment ($P = 0.001$). At adjusted analysis, prolonged duration of treatment was independently associated with lower ($P = 0.006$) and undetectable ($P < 0.001$) HIV DNA values.

The assessment of cell-associated human immunodeficiency virus type 1 (HIV-1) DNA is a useful tool to study the impact of highly active antiretroviral therapy (HAART) on the cell-associated reservoir in HIV-infected patients (1). The cellular HIV-1 DNA load in peripheral blood mononuclear cells (PBMCs), independently of HIV RNA and CD4 cell count, is associated with progression to AIDS and death (2, 3, 6, 9). The pre-HAART HIV DNA concentration was a predictor of short- and long-term success of therapy in hemophiliac patients (6) and of the level of residual viremia after HAART (5, 12). This cross-sectional study was designed to investigate demographic, virological, and immunological determinants of low-level cellular HIV DNA in HIV-infected patients with persistently undetectable plasma viremia (<50 copies HIV RNA/ml) during HAART.

We selected 163 HIV-infected subjects at several Italian institutions on the following characteristics: receiving their first or second HAART regimen for more than 8 months; having persistent undetectable viremia; no virological failure; being ≥ 18 years old; having a high self-reported adherence; and having provided informed consent.

Total cell DNA was extracted from pellets by the High Pure PCR template preparation kit (Roche Diagnostics GmbH, Germany); HIV-1 cellular DNA in PBMCs was quantified by real-time PCR according to the Real-Time TaqMan protocol (11), adapted to the LightCycler (Roche Molecular Biochemicals, Indianapolis, IN). The primers amplified integrated as well as unintegrated HIV DNA.

Chi square and Kruskal-Wallis tests were used to assess differences between patient groups. To obtain an adjusted analysis we used a multiple logistic regression model. All the analyses are intention-to-treat using SPSS (SPSS Inc., Chicago, Illinois). A standard curve was designed using seven serial dilutions of 8E5 cell DNA. Comparison of the test samples with this standard curve was used to quantify cellular HIV-1 DNA with sensitivity of up to 20 copies/10⁶ PBMCs.

The median age of the 163 selected patients was 38.5 years (range, 24 to 77 years), 54 subjects were female (33%), 62 subjects (38%) reported having sex with men as a risk factor for HIV, 81 patients (49.6%) were heterosexual, and 18 patients (11%) were intravenous drug users. At the beginning of HAART the median CD4 count was 343 cells/mm³ (range, 2 to 1,030 cells/mm³) and the median HIV RNA level was 4.76 copies/ml (range, 2.34 to 6.3 copies/ml).

Current HAART included two nucleoside reverse transcriptase inhibitors (NRTIs) plus a protease inhibitor (PI) in 72 subjects (44%), 19 of them treated with a boosted PI, or plus a non-NRTI (NNRTI) in 78 subjects (48%), and 13 patients (8%) were treated with three NRTIs. The current regimen represented the first-line therapy in 80 subjects (49%), 36 of them treated with a PI-based regimen, 42 with an NNRTI-based regimen, and 2 with three NRTIs. One hundred and nine patients (67%) were PI exposed and 101 patients (62%) were NNRTI exposed.

All patients were treated with HAART for a median of 25 months (range, 8 to 93 months) and the current median CD4 count was 618 cells/mm³ (range, 94 to 1,686 cells/mm³). Detectable levels of HIV DNA in PBMCs were found in all but 15 patients (91%) with a median value of 360 copies/10⁶ PBMCs (range, < 20 to 11,444 copies/10⁶ PBMCs). Patients with HIV

* Corresponding author. Mailing address: Department of Public Health and Cellular Biology, University of Tor Vergata, Via Montpellier 1, 00133 Rome, Italy. Phone and fax: (39-06) 72596873. E-mail: andreoni@uniroma2.it.

TABLE 1. Characteristics of 163 patients above and below the 25th percentile of cellular HIV DNA copies (133 copies/10⁶ PBMCs)

Parameter	Mean HIV DNA copy number/ 10 ⁶ PBMCs $\pm$ SD		<i>P</i>
	≤ 133 copies (<i>n</i> = 44)	> 133 copies (<i>n</i> = 119)	
Age (yr)	39.2 $\pm$ 8.8	41.9 $\pm$ 11.1	0.15
No. of patients (%)			0.3
Female	12 (27.2)	42 (35.3)	
Male	32 (72.8.4)	77 (64.7)	
No. (%) of patients with HIV risk factor:			0.06
Heterosexual relationship	16 (36.3)	65 (54.6)	
Homosexual relationship	24 (54.6)	38 (31.9)	
Injection drug use	4 (9.1)	14 (11.8)	
Other	0	2 (1.7)	
Pre-HAART CD4 cell count (cells/mm ³)	446 $\pm$ 281	311 $\pm$ 212	0.001
Pre-HAART CD4 cells (%)	24.0 $\pm$ 11.3	17.9 $\pm$ 10.0	0.002
Pre-HAART HIV RNA (log ₁₀ copies/ml)	5.2 $\pm$ 5.5	5.3 $\pm$ 5.5	0.58
Current CD4 cell count (cells/mm ³)	764 $\pm$ 350	604 $\pm$ 310	0.005
Current % CD4	34.8 $\pm$ 11.6	29.6 $\pm$ 11.2	0.01
CD4 cell count recovery during HAART (cells/mm ³)	317 $\pm$ 241	292 $\pm$ 206	0.51
Duration of HAART (mo)	33.5 $\pm$ 17.0	25.3 $\pm$ 12.6	0.001
No. (%) of patients on HAART for:			0.01
<12 mo	1 (2.3)	8 (6.7)	
12–24 mo	13 (29.5)	58 (48.7)	
24–36 mo	16 (36.4)	39 (32.8)	
>36 mo	14 (31.8)	14 (11.8)	
PI exposure (no. [%] of patients)			0.87
Naive	15 (34.1)	39 (32.8)	
Exposed	29 (65.9)	80 (67.2)	
NNRTI exposure (no. [%] of patients)			0.92
Naive	17 (38.6)	45 (37.8)	
Exposed	27 (61.4)	74 (62.2)	
Current HAART (no. [%] of patients)			0.6
PI-based	19 (43.2)	53 (44.5)	
NNRTI-based	20 (45.4)	58 (48.7)	
3 NRTIs	5 (11.4)	8 (6.8)	

DNA copies below the 25th percentile (133 copies/10⁶ PBMCs) more frequently reported unprotected homosexual behavior ($P = 0.06$), higher pre-HAART CD4 cell count and percentage ($P = 0.001$ and $P = 0.002$, respectively), higher current CD4 cell count and percentage ($P = 0.005$ and $P = 0.01$), and prolonged duration of antiretroviral treatment ($P = 0.001$) compared to other patients (Table 1). At logistic regression analysis, the duration of treatment was independently associated with lower HIV DNA values (odds ratio, 0.48; 95% confidence interval, 0.28 to 0.81; $P = 0.006$).

Figure 1 shows the distribution of cellular HIV DNA copy numbers according to time in months after initiation of HAART. No patients treated for more than 36 months with HAART had cellular HIV DNA levels above 1,500 copies/10⁶ PBMCs; 5 out of 11 patients (45.4%) treated for more than 48 months had an undetectable level of cellular DNA, whereas

among 79 patients treated for less than 24 months only one patient (1.2%) had undetectable HIV DNA.

No significant differences in gender, HIV risk factors, pre-HAART and current CD4 cell count, or pre-HAART HIV RNA level were found among the 15 patients with an undetectable HIV DNA load and the subjects with detectable levels (data not shown). A significantly longer period of antiretroviral treatment was observed in patients with undetectable HIV DNA load ($P < 0.0001$). These patients were treated with maximally successful HAART with persistent undetectable plasma viremia for 46.8 months (median, 39 months; range, 24 to 85 months). At logistic regression analysis, the duration of treatment was confirmed to be independently associated with undetectable HIV DNA values (odds ratio, 0.16; 95% confidence interval, 0.06 to 0.43; $P < 0.001$).

In this cross-sectional study 163 patients with persistently undetectable HIV RNA viremia during HAART had variable amounts of cellular HIV DNA. Unprotected homosexual behavior, high pre-HAART and current CD4 cell counts, and prolonged periods of antiretroviral treatment were parameters more commonly observed in patients with levels of cellular DNA below the 25th percentile. No difference in terms of antiretroviral drug exposure with respect to HIV DNA levels was observed. At logistic regression analysis only the length of antiretroviral treatment was independently associated with low HIV DNA levels. The inverse correlation between years spent on HAART and amount of cellular DNA in PBMCs is not surprising, since in patients with a median HAART exposure of 25 months, HIV DNA is likely to still be in a decay phase.

In previous studies the rate of decline of HIV DNA load was clearly associated with the long-term success of HAART (4, 7, 8, 10). During HAART the kinetics of cell-associated DNA presents a two-phase decay characterized by a rapid decay rate in the first 3 months followed by a slower decay rate, corresponding to a mean half-life of 6.6 months (12). This second-phase DNA decay is characterized by large interindividual

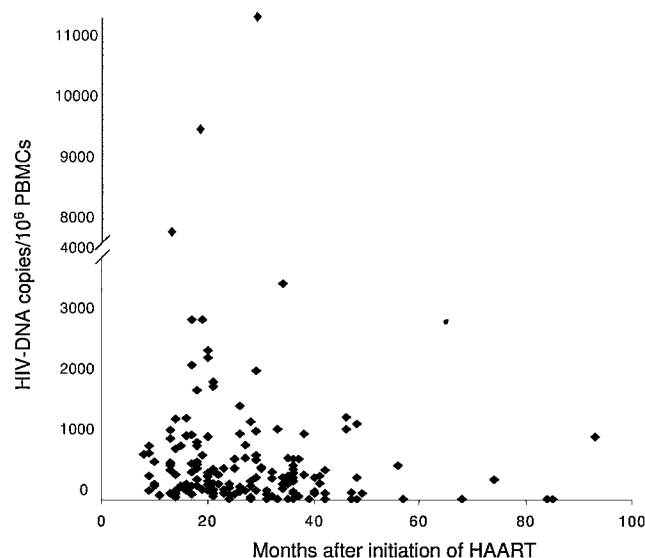


FIG. 1. Distribution of cellular HIV DNA copies according to months after initiation of HAART in 163 patients with undetectable plasma HIV RNA loads.

differences and is strongly correlated with the mean level of cell-associated RNA in PBMCs. Consistent with this report (12), in our study 15 out of 163 patients (9.2%) reached undetectable levels of HIV DNA in PBMCs. All these patients were treated with maximally successful HAART for at least 2 years (range, 24 to 85 months). Conversely, a recent report found that patients with sustained undetectable HIV viremia during HAART experienced a progressive decrease of HIV DNA until the third year without any further diminution afterwards (11). However, a different method was used to analyze HIV DNA and only 25 patients were enrolled.

In our study, the HIV DNA decay could be mainly referred to unintegrated DNA. In fact, it seems plausible that new replication events that sustain unintegrated DNA were better suppressed in patients with longer durations of therapy than that integrated copies of HIV DNA declined over a relatively short period of time. Nevertheless, nearly 10% of the patients reached undetectable cellular HIV DNA levels.

We are aware of the limitations of our study, mainly represented by its observational nature, the relatively small number of patients, and the underrepresentation of boosted PI regimens. Nevertheless, the observed association between low cellular DNA levels and length of antiretroviral treatment may have implications for the choice of initial antiretroviral treatment and for clinical strategies. It is difficult to imagine treatments as life-long prescriptions, given the side effects described in the long term. In this scenario, the HIV DNA level can help to identify patients for whom the strategy of simplified maintenance therapy or structured interruption might be revisited.

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