Relationships between HIV disease history and blood HIV-1 DNA load in perinatally infected adolescents and young adults: The ANRS-EP38-IMMIP Study

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Abstract

Background: Our aim was to study the impact of lifelong HIV disease history on the current immune and virological status of perinatally infected patients reaching adulthood. We evaluated blood cell-associated HIV DNA load, as an indicator of cell-associated HIV reservoirs and an independent predictor of disease progression.

Methods: The ANRS-EP38-IMMIP Study included 93 patients between the ages of 15 and 24 years, who were infected with HIV during the perinatal period. HIV DNA load was quantified by real-time PCR.

Results: Eighty-five percent of patients were on HAART, and HIV RNA was undetectable in the plasma of 75% of these patients. The median HIV DNA load was 2.84 (IQR: 2.51-3.16) $\log_{10}$ copies/10^6 PBMCs. In patients with viral suppression, HIV DNA load was independently associated with cumulative HIV RNA viremia over the last five years. HIV DNA load was negatively correlated with CD4 cell count in patients with active replication but not in those with undetectable HIV RNA.

Conclusions: In perinatally infected youths who are successfully treated, sustained viral suppression is associated with low HIV DNA load. The absence of association between current HIV DNA load and CD4 cell counts suggests that the unique physiological characteristics of pediatric infection persist after adolescence.

Keywords: HIV perinatal infection, adolescents, cell-associated HIV DNA, cumulative viremia

Highly active anti-retroviral therapy (HAART) has transformed HIV disease into a stable chronic condition. Infected children born at the beginning of the epidemic are now reaching adulthood [1]. The many specific features of pediatric HIV disease preclude an extrapolation from data gathered in adults. In the absence of treatment, infected children have a high level of viral replication that persists for several years, and their immune system develops in the presence of an immunosuppressive virus [2-3]. This heavy exposure to deleterious viral replication is counterbalanced by high thymic activity that provides a better replenishment of CD4 T-lymphocytes than in infected adults [4-5]. The unique characteristics of perinatal infection described in children may be altered by long-term exposure to viral replication, various antiretroviral (ARV) treatments and pubertal development. Improvements in our knowledge of the virological and immunological status of these patients, who are now approaching adulthood is required, as these characteristics are likely to affect future clinical progression.

In the French national perinatal cohort (ANRS-CO-10), 60% of the children born before 1994 and included at birth are still alive and regularly followed-up [6]. The first generation of HIV-1-infected children did not benefit from either the prophylaxis of vertical transmission or from early access to HAART. Many received nucleoside inhibitors of reverse transcriptase (NRTI) monotherapy as their initial treatment. In 2007, we set up the ANRS-EP38-IMMIP Study to focus on HIV-1 infected youths over the age of 15 years, who had been infected during the perinatal period. Through this study, we aimed to improve our knowledge of virological and immune status of these patients, by assessing biological parameters not evaluated in routine clinical practice and identifying correlations between these parameters and life-long HIV disease parameters.

Cell-associated HIV DNA load reflects the viral reservoir, whereas plasma HIV RNA load quantifies active viral replication [7]. Independently of HIV RNA load and CD4 cell

counts, blood HIV DNA load is predictive of disease progression [8-9]. In the absence of quantifiable viral replication, it is the only virological marker available and is predictive of residual replication and immune reconstitution in treated adults [10-13]. In children, HIV DNA load is associated with clinical status, plasma viral load, CD4 cell counts, immune activation and HIV-specific immune responses [14-17]. In children initiating HAART, a higher baseline HIV DNA level is associated with a longer time lag to undetectable HIV RNA levels and with virological failure [18-19]. The results of studies of immune restoration under HAART differ considerably between pediatric and adult patients: HIV DNA levels have been associated with a limited increase in the number of naive CD4 lymphocytes in adults, but with higher thymic output in children [12-13, 18, 20]. We studied HIV DNA load and its relationship to lifetime HIV disease history, 15 years or more after perinatal infection.

Patients and methods

Study design

Between February 2007 and February 2009, 93 HIV-1-infected patients followed up at 10 clinical sites within the Paris area were enrolled in the ANRS-EP38-IMMIP Study. The inclusion criteria were (1) HIV-1 infection through the vertical route, (2) being over the age of 15 years, (3) absence of change in therapeutic status during the previous six months, (4) initiation into care before 1996, to ensure that all patients had similar access to HAART. This study was approved by the local ethic committees. Patients and their legal guardians signed an informed consent form. All but one of the patients were born in France. Sixty-eight patients were included at birth in the ANRS-CO-10 cohort [6]. Their current HAART status, HIV RNA load and CD4 cell counts were not significantly different from those of the other 25 perinatally infected patients.

Biological evaluations at the time of the study

A single 25 ml blood sample was taken for biological evaluations. HIV DNA load was quantified in leukocytes by real-time PCR (ANRS assay, Biocentric, Bandol, France) carried out at a single laboratory, for 91 patients [21]. No results were available for the remaining 2 patients, due to PCR inhibition. The detection threshold was 1.5 $\log_{10}$ HIV DNA copies/10^6 leukocytes. The detection threshold of HIV DNA/10^6PBMCs depended on leukocyte, lymphocyte and monocyte counts. CD8 T lymphocyte count was determined on fresh whole blood, using a combination of the following antibodies: CD45-FITC, CD4-RD1, CD8-ECD, CD3-PC5 and CD19-PC7 (Beckman-Coulter, Villepinte, France). Labeled cells were analyzed on a FC500 cytometer (Beckman-Coulter).

HIV history variables

Clinical stage was defined according to the CDC classification [22-23]. HAART was defined as any combination of at least three different ARV drugs or any combination including either one protease inhibitor or one non-nucleoside reverse transcriptase inhibitor. CD4 cell count and percentage, and plasma HIV RNA level were quantified at the clinical sites, as part of the standard follow-up procedure. At the time of the study, the threshold values used in HIV RNA assays depended on the volume of plasma available. The highest threshold used was 80 copies/ml, and this value was selected as the cutoff for current HIV RNA detection. A median of 42 (IQR: 34-51) CD4 cell percentage determinations and a median of 32 (IQR: 27-39) HIV RNA measurements were collected over the lifetime of each patient. The 500 copies/ml cutoff was chosen for assessments of virological history, due to changes in HIV RNA quantification assays over time. Cumulative viremia was defined as the area under the curve of HIV RNA load over time and was estimated as described by Zoufaly *et al.* [24]. Immunological history parameters were defined using values of CD4 cell percentages because age-related variations in this parameter are smaller than those of CD4 cell counts. Variables relating to HIV disease history were assessed for the entire follow-up

period and for two other time periods. The last 10 years of follow-up was the longest period over which all patients had access to HAART and HIV RNA quantification. We also assessed the last five years of follow-up, to describe recent HIV disease history.

Statistical analysis

We studied univariate associations between HIV DNA load in PBMCs and variables defining current and past HIV disease, using the Wilcoxon test for categorical variables, and the Spearman's rank or Pearson's correlation coefficients for continuous variables. Univariate and multivariate analyses were performed by linear regression, with HIV DNA load as the continuous dependent variable. We adjusted for noncollinear variables with $P < 0.20$. Analyses were conducted with SAS statistical software (version 9.2). $P < .05$ defined statistical significance.

Results

Characteristics of patients

Current status

The 93 patients included in the ANRS-EP38-IMMIP Study were born between January 1983 and February 1994, and were between 15 and 24 years of age at the time of the study. Their current and past characteristics are presented in Table 1. The mothers of about half of the patients originated from mainland France, whereas those of about 30% originated from Sub-Saharan Africa, the mothers of the remaining 20% originated from the Caribbean, North Africa, or Asia. Seventy-nine (85%) patients were receiving HAART at the time of the study. Ten of the 14 untreated patients had previously been on HAART and four had never received HAART. The four HAART-naive patients had a median of 445 CD4 cells/µl, a median plasma viral load of 3.4 $\log_{10}$ HIV RNA copies/ml, and they were at CDC stages N or A. Three of the 93 patients had a CD4 cell count below 200 cells/µl and 11 had a CD4 cell percentage below 15%. HIV RNA was undetectable in the plasma of 75% of the treated

patients and in none of the 14 untreated patients (P<0.0001). Median CD4 cell counts were 612 cells/µl in treated patients, and 422 cells/µlin untreated patients (P=0.004).

Lifetime HIV disease and treatment

Throughout their lifetimes, 13% of the patients had never presented HIV-related symptoms (CDC stage N), whereas 27%, 35% and 25% of patients had reached CDC stages A, B and C, respectively (Table 1). At time of the first CDC stage C clinical event, seven of the patients were less than one year old and three were between one and two years of age. All patients had received at least one course of ARV treatment, initiated at a median age of one (IQR: 0-2) year for a median cumulative duration of 14 (IQR:11-16) years (Table 1). NRTI monotherapy had been prescribed to 89% of patients and NRTI bitherapy to 87%. Only one child received zidovudine for postnatal prophylaxis. At the time of HAART initiation, only five (5.4%) patients had never been treated with ARV drugs. Median age at the first course of HAART was seven (IQR: 5-10) years and the median cumulative duration of HAART was nine (IQR: 7-11) years. Thirty-two out of the 89 patients (36%) who had initiated HAART had never interrupted it at the time of the study.

Since birth/start of follow-up, CD4 cell percentage had been below 25% at least once in all but one of the patients and below 15% at least once in 80% of the patients. Median times for which CD4 cell percentage were below 25% and 15% were 7.8 (IQR: 4.6-11.9) years and 1.9 (IQR: 0.5-4.1) years, respectively. Median zenith value of plasma viral load was 5.2 (IQR: 4.9-5.5) $\log_{10}$ HIV RNA copies/ml. Median cumulative period for which HIV RNA was below 500 copies/ml was 5.6 (IQR: 2.8-7.6) years. The most recent period for which HIV RNA was continuously below 500 copies/ml was much shorter (median (IQR): 1.6 (0-4.8) years).

Relations between current HIV DNA load and HIV disease history

The median HIV DNA load in PBMCs was 2.84 (IQR: 2.51-3.16) log_{10} copies/10^6 PBMCs. The HAART-naive patients had HIV DNA loads ranging from 2.65 to 3.30 log_{10} copies/10^6 PBMCs (Figure 1). Patients with HAART interruption and treated patients with detectable HIV RNA had the highest HIV DNA loads (ranges: 2.57-3.58 and 1.82-4.08 log_{10} copies/10^6 PBMCs, respectively). The patients receiving HAART with undetectable HIV RNA had the lowest HIV DNA loads (range: <1.9-3.54 log_{10} copies/10^6 PBMCs). The HIV DNA loads of patients with undetectable HIV RNA were significantly lower than those of patients with HAART interruption and treated patients with detectable HIV RNA (*P*=0.009 and *P*=0.0007, respectively) and not significantly different from the one of HAART-naive patients (*P*=0.20).

Univariate analysis of associations between the current HIV DNA load and the current status

HIV DNA load was not associated with age, maternal geographic origin or CDC stage (Table 2). HIV DNA load tended to be higher in girls than in boys (*P*=0.06). HIV DNA load was significantly lower in treated than in untreated patients (*P*=0.04). It was positively correlated with both HIV RNA load (r=0.526, *P*<0.0001) and CD8 cell count (r=0.302, *P*=0.008), and negatively correlated with CD4 cell count (r=-0.327, *P*=0.001) (Figure 1).

Analyses were stratified on the basis of current HAART and HIV RNA detection (Table 2). HIV DNA load was positively correlated with HIV RNA load in untreated and treated patients with detectable viremia (r=0.609, *P*=0.03 and r=0.761, *P*=0.0002, respectively). It was negatively correlated with CD4 cell counts in treated patients with detectable viremia (r=-0.790, *P*<0.0001). By contrast, HIV DNA load was not correlated with CD4 cell count in patients with undetectable viremia (r=0.017, *P*=0.89). There was a significant interaction between HIV RNA detection and CD4 cell count (*P*=0.01).

Univariate analysis of associations between current HIV DNA load and HIV disease history

HIV DNA load was negatively correlated with both the cumulative duration of HAART (r=-0.297, *P*=0.004) and the duration of HIV RNA suppression to levels below 500 copies/ml (r=-0.513, *P*<0.0001) over the last five years of follow-up. Similar results were obtained if we considered the last 10 years of follow-up (Table 2). The positive correlation between HIV DNA load and cumulative viremia was slightly stronger for the last five years period than for the last 10 years (r=0.524, *P*<0.0001 and r=0.474, *P*<0.0001) and for the two- and three-years periods (data not shown). Stratified analysis confirmed that virological history was associated with current HIV DNA load (Table 2). Regardless of current HAART or HIV RNA status, HIV DNA load was correlated (or there was a trend toward correlation) with the length of time for which HIV RNA load was below 500 copies and cumulative viremia. HIV DNA load was not associated with age, CD4 cell percentage, HIV RNA load at HAART initiation, numbers of HAART interruptions, HIV RNA zenith levels, CD4 percentage nadir levels, or the duration of severe immunosuppression (Table 2).

Multivariate analysis

As there was a significant interaction between the detection of HIV RNA and the CD4 cell count, we performed separate multivariate analysis in patients with detectable viral load and in those in whom HIV RNA was undetectable (Table 3). In patients with detectable HIV RNA, whether untreated or receiving HAART, HIV DNA load was independently associated with current HAART (*P*=0.03), HIV RNA load (*P*=0.02) and CD4 cell counts (*P*=0.02), after adjustment for age, sex and cumulative viremia over the last five years. In treated patients with undetectable HIV RNA, HIV DNA load was independently associated with cumulative viremia over the last five years (*P*=0.02) after adjustment for age, sex and CD4 cell count.

Discussion

This work focused HIV-1-infected young adults and adolescents older than 15 years who had been infected perinatally with HIV-1. Most of these patients had reached advanced stages of disease: 80% had had at least one CD4 cell percentage below 15% and only four of them never received HAART. Of note, 10 out of 93 patients reached the CDC stage C before the age of two. We present the first description of blood HIV DNA load and its association with HIV disease history in these patients. We showed that in patients with currently undetectable HIV RNA, cumulative viremia is the only factor associated with HIV DNA load. Furthermore, the association between HIV DNA load and CD4 cell counts differed between patients with active and suppressed viral replication.

Median HIV DNA load was 2.8 $\log_{10}$ copies/10^6 PBMCs in treated youths with undetectable HIV RNA, similar to that reported for chronically infected adults treated in the early HAART period [25-26]. Lower HIV DNA loads, around 2.2-2.3 $\log_{10}$ copies/10^6 PBMCs, were reported in adults who had recently initiated HAART (i.e. in 2003 or later), with a moderate baseline HIV RNA load and who had either never received ARV drugs or had a CD4 cell count >350 cells/µl at baseline [27-28]. Adult patients beginning HAART at disease stages similar to that of our study population had HIV DNA loads of 2.2 and 2.5 $\log_{10}$ copies/10^6 PBMCs after 10 and three years of permanently undetectable HIV RNA, respectively [29]. In our study population, 64% of the patients interrupted HAART at least once, and the median time for which HIV RNA was below 500 copies/ml before this study was 1.6 years. Thus, taking into account the long duration of HIV infection and complex HAART history of these perinatally infected youths, their HIV DNA load was similar to that expected for adult patients.

Our results suggest that once the viral replication is suppressed, the previous viral history is the major factor associated with current cell-associated HIV DNA load. In early reports, HIV DNA levels were shown to decline over a limited period, ranging from nine

months to three years, before reaching a plateau [25, 30-31]. In our patients with viral suppression, cell-associated HIV DNA load was correlated with cumulative exposure to viral replication over longer periods of time. Another recent cross-sectional study performed in adults also suggested that sustained virological suppression over a decade was associated with low HIV DNA load [29].

The importance of cumulative exposure to viral replication as a predictor of clinical progression has been highlighted in recent studies [24, 32]. The interest of such cumulative parameters will undoubtedly increase as patients live longer, as in this study. Cumulative viremia is partly influenced by the HAART-induced suppression of viral replication. Indeed, the duration of HAART and the duration of viral suppression were found to be associated with HIV DNA load, although less strongly than cumulative viremia (Table 2). The major advantage of considering cumulative viremia is that it takes into account both the duration of periods with undetectable and detectable HIV RNA and the level of viral replication in the latter ones. During periods of detectable viral replication, cumulative viremia summarizes all factors that influence HIV RNA level: the partial efficacy of former therapeutic regimens, periods of suboptimal adherence with treatment, the properties of the virus, the immune response and other host factors.

One key finding of this study is that the association between current HIV DNA load and CD4 cell counts is affected by the suppression of viral replication. In patients with active viral replication, HIV DNA load was independently correlated with CD4 cell count and HIV RNA load, as previously reported for pediatric [16, 18] and adult patients [33-34]. By contrast, HIV DNA load is not associated with CD4 cell counts in patients with suppressed viremia. This probably reflects the marked quantitative and qualitative differences in immune recovery between children and adults. Indeed, in a longitudinal follow-up of children initiating HAART, an inverse correlation between HIV DNA load and CD4 cell counts was

observed at baseline, but ceased to be significant as little as eight weeks after treatment initiation [15]. Another pediatric study showed that the decline in HIV DNA levels and the increase in CD4 cell counts were correlated after one month of HAART, but not during subsequent periods of follow up [31]. The results obtained for perinatally infected patients differed considerably from those in adults, in whom HIV DNA load is negatively correlated with current and nadir CD4 cell counts in the absence of viral replication [26, 33-34]. In addition, restoration of the CD4 lymphocyte population depends principally on the generation of naive CD4 lymphocytes in children and the expansion of the memory CD4 lymphocyte population in adults [18, 35-37]. Furthermore, the absence of correlation between HIV DNA level and CD4 cell counts in our patients may reflect differences in the dynamics of cell-associated virus levels and CD4 cell counts in response to successful HAART. HIV DNA level declines during the first one to three years after HAART initiation [25, 30-31] whereas CD4 cell counts increase over longer periods [38-39]. As the patients received HAART for a median of nine years, the differences in HIV DNA level and CD4 cell counts dynamics may be responsible for the loss of correlation between the two parameters.

The major strength of our study is the very thorough documentation of HIV disease history in patients with a known date of infection and more than 15 years of follow up. The patients from the ANRS-EP38-IMMIP Study had demographic characteristics very similar to those of patients from ANRS-CO-10 cohort born before 1994, but with a higher proportion of patients with undetectable HIV RNA [6]. This may be due to the application of the inclusion criterion requiring patients to have had no change in treatment status for at least six months. This inclusion criterion probably had only a very minor impact on associations between HIV DNA load and disease history, as the median duration of HAART was nine years. Another limitation is the small number of patients in the HAART-naive and HAART-interruption groups, precluding independent analysis. One limitation of our work was that HIV RNA was

quantified since 1996 only. Thus, virological history was known for the entire HAART period for each patient, but not since birth. French regulations limit the amount of blood that can be drawn from individual patients. This precluded the quantification of other potentially relevant viral markers, such as integrated HIV DNA load or HIV RNA load determination in a single-copy assay. Determination of the total HIV DNA load in PBMCs has the major advantage of requiring only a small volume of blood. This marker reflects the blood HIV reservoir and it has frequently been identified as a relevant biomarker for the management of HIV-1-infected patients [10-11, 16, 27-28].

This study shows that perinatally infected patients with regular care can achieve a good virological/immunological status, even after reaching advanced disease stages and being heavily ARV-treated for more than a decade. In successfully treated patients, viral replication history is a major determinant of cell-associated HIV DNA load. Most of the patients in this study began HAART during childhood and had reached adolescence or adulthood at the time of the study. Their HIV DNA loads were similar to those reported in adults with a similar disease history. However, their CD4 cell counts appeared to be slightly higher than those of adults with long-term HIV infection [26, 40-41]. Furthermore, we report the absence of a correlation between HIV DNA load and CD4 cell counts when viral replication is suppressed, suggesting that the interactions between HIV replication and the immune system are specific in pediatric infection. Overall, our work shows that infection during the perinatal period has a long-term impact on the immune and virological status of HIV infection resulting in differences with respect to patients infected during adulthood.

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APPENDIX

This study was approved by "the Comité de protection des personnes Ile-de-France II" (registration number 06-09-08), was authorized by the "Direction Générale de la Santé" (authorization number 2006-AOO142-49), and was registered as an observational study at www.clinicaltrials.gov under identifier NCT01055873.

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Table 1: Characteristics of patients included in the ANRS-EP38-IMMIP Study

Characteristic	
Age [a]	
median [IQR]	17 [15-19]
Sex, % (n)	
Male	43.0 (40)
Female	57.0 (53)
Maternal geographic origin, % (n)	
Mainland France	50.5 (47)
North Africa	8.6 (8)
Sub-Saharan Africa	30.1 (28)
Caribbean	8.6 (8)
Asia	1.1 (1)
Unknown (adoption)	1.1 (1)
Current HAART[b] at the time of the study, % (n)	
No	
HAART-naive	4.3 (4)
HAART interrupted	10.8 (10)
Yes	84.9 (79)
Current status	
CD4 cell count/µl, median [IQR]	581 [418-781]
CD4 cell percentage, median [IQR]	29 [22-34]
HIV RNA [c], median [IQR]	1.4 [1-3.1]
CDC stage [d], % (n)	
N	12.9 (12)
A	26.9 (25)
B	35.5 (33)
C	24.7 (23)

Table 1, continued

Treatment during lifetime	
ARV [e] (all types)	
% (n)	100.0 (93)
Age at first ARV [a], median [IQR]	1 [0-2]
Cumulative duration [a,f], median [IQR]	14 [11-16]
1 NRTI [g]	
At least one sequence, % (n)	89.2 (83)
Cumulative duration [a,f], median [IQR]	3 [2-4]
2 NRTI [g]	
At least one sequence, % (n)	87.1 (81)
Cumulative duration [a,f], median [IQR]	1 [1-3]
HAART	
At least one sequence, % (n)	95.7 (89)
Age at first HAART [a], median [IQR]	7 [5-10]
Cumulative duration [a,f], median [IQR]	9 [7-11]
CD4 history during lifetime	
CD4 percentage < 25, % (n)	
Never	1.1 (1)
At least once	98.9 (92)
CD4 percentage < 15, n (%)	
Never	20.4 (19)
At least once	79.6 (74)
Cumulative duration of CD4 cell percentage < 25 [a], median [IQR]	7.8 [4.6-11.9]
Cumulative duration of CD4 cell percentage < 15 [a], median [IQR]	1.9 [0.5-4.1]
CD4 cell count nadir [h], median [IQR]	160 [52-302]
CD4 cell percentage nadir [h], median [IQR]	8 [3-13]
HIV RNA during lifetime	
Zenith HIV RNA [c,h], median [IQR]	5.2 [4.9-5.6]
Cumulative duration of HIV RNA < 500 copies/ml [a], median [IQR]	5.6 [2.8-7.6]
Duration of last period of HIV RNA < 500 copies/ml [a], median [IQR]	1.6 [0-4.8]

[a] expressed in years, [b] HAART, highly active antiretroviral treatment, [c] expressed as $\log_{10}$ HIV RNA copies/ml of plasma, [d] according to the Centers for Disease Control and Prevention classification [22-23], [e] ARV, antiretroviral treatment, [f] cumulative durations of treatment

were calculated without taking into account treatment interruptions of less than 15 days, [g] NRTI: nucleoside reverse transcriptase inhibitor, [h] Nadir CD4 cell percentage and zenith HIV RNA values were defined as the lowest and highest value reached during follow up, respectively.

Table 2: Univariate analysis of factors associated with HIV DNA load in PBMCs

	All patients		Stratified according current detectable HIV-RNA and therapeutic status					
			Untreated		HAART, Detectable HIV RNA n=19		HAART Undetectable HIV RNA	
	n=91		n=13				n=59	
	Median (IQR)	*P* [a]	Median (IQR)	*P* [a]	Median (IQR)	*P* [a]	Median (IQR)	*P* [a]
Current status								
Sex								
Female	2.89 (2.61-2.91)	0.06	3.22 (2.78-3.48)	0.37	3.23 (3.07-3.80)	0.21	2.81 (2.20-3.00)	0.15
Male	2.79 (2.26-2.66)		2.87 (2.57-3.22)		3.02 (2.90-3.44)		2.53 (2.51-3.04)	
Maternal geographic origin								
Sub-Saharan Africa	2.87 (2.54-3.21)	0.47	2.97 (2.78-3.26)	0.94	3.17 (3.07-3.66)	0.49	2.51 (2.20-2.82)	0.27
Others	2.84 (2.49-3.10)		3.22 (2.77-3.44)		3.02 (2.66-3.39)		2.79 (2.46-3.04)	
CDC stage C								
No	2.80 (2.49-3.11)	0.41	3.01 (2.78-3.30)	0.70	3.07 (2.91-3.67)	0.87	2.68 (2.38-2.88)	0.14
Yes	2.94 (2.54-3.23)		3.41 (2.73-3.48)		3.23 (2.55-3.44)		2.92 (2.51-3.05)	
Current HAART								
No	3.15 (2.78-3.41)	0.04	n.a.[b]			n.a.[b]		n.a.[b]
Yes	2.83 (2.48-3.09)							
	r [c]	*P* [c]	rho [c]	*P* [c]	rho [c]	*P* [c]	r [c]	*P* [c]
Age	-0.042	0.69	-0.071	0.81	-0.192	0.43	0.055	0.68
CD4 cell count	-0.327	0.001	-0.209	0.49	-0.790	<0.0001	0.017	0.89
CD8 cell count	0.302	0.008	0.297	0.41	0.137	0.60	0.155	0.28
Plasma HIV RNA [d]	0.526	<0.0001	0.609	0.03	0.761	0.0002	n.a.[d]	

Table 2 (continued)	r [c]	*P* [c]	rho [c]	*P* [c]	rho [c]	*P* [c]	r [c]	*P* [c]
HAART history								
Cumulative duration of HAART over the last 5 years	-0.297	0.004	0.350	0.24	-0.396	0.13	-0.247	0.06
Cumulative duration of HAART over the last 10 years	-0.297	0.004	0.172	0.57	-0.315	0.19	-0.226	0.08
Number of HAART interruptions	-0.089	0.41	0.000	1	-0.046	0.85	-0.150	0.25
Age at initiation of first HAART	0.138	0.20	0.481	0.19	0.153	0.53	0.093	0.48
CD4 history								
CD4 cell percentage at initiation of first HAART [e]	0.114	0.32	0.453	0.26	-0.177	0.48	0.193	0.17
CD4 cell percentage nadir [f]	0.024	0.82	-0.450	0.12	-0.181	0.46	0.194	0.14
Cumulative duration of CD4% < 15	0.048	0.65	0.295	0.33	0.124	0.61	0.181	0.17
HIV RNA history								
HIV RNA at initiation of first HAART [d, e]	0.052	0.66	0.033	0.93	-0.096	0.72	0.048	0.74
Cumulative duration of HIV RNA < 500 copies/ml over the last 5 years	-0.513	<0.0001	-0.126	0.68	-0.621	0.005	-0.352	0.007
Cumulative duration of HIV RNA < 500 copies/ml over the last 10 years	-0.510	<0.0001	-0.341	0.26	-0.547	0.02	-0.332	0.01
HIV RNA zenith [d,f]	-0.057	0.59	0.377	0.20	0.114	0.64	-0.208	0.11
Cumulative viremia over the last 5 years [g]	0.524	<0.0001	0.450	0.12	0.338	0.009	0.281	0.03
Cumulative viremia over the last 10 years [g]	0.474	<0.000	0.489	0.09	0.524	0.02	0.196	0.13

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[a] For categorical variables, Wilcoxon test's *P* value are reported, [b] n.a., not applicable, [c] For groups with > 30 patients, Pearson's correlation coefficient and *P* value means are reported; for groups with ≤ 30 patients, Spearman's rho and *P* value are reported, , [d] expressed as $\log_{10}$ HIV RNA copies/ml of plasma, [e] pretherapeutic CD4 cell percentage and HIV RNA loads were defined as the most recent measurement within the six months preceding HAART initiation, [f] Nadir CD4 cell percentage and zenith HIV RNA values were defined as the lowest and highest values reached during follow up, respectively, [g] expressed as days x $\log_{10}$ HIV RNA copies/ml of plasma.

Table 3: Multivariate analysis of factors associated with HIV DNA load

		Unadjusted analysis [a]			**Adjusted analysis** [b]	
	Estimate	[95% CI]	*P*	Estimate	[95% CI]	*P*
Model 1 (Patients with detectable HIV RNA)						
Sex (Female versus Male)	0.26	[-0.10;0.63]	0.15	0.18	[-0.09;0.45]	0.17
Current HAART (Yes versus No)	0.04	[-0.33;0.42]	0.81	0.29	[0.03;0.54]	0.03
Age [c]	-0.03	[-0.11;0.05]	0.42	-0.03	[-0.08:0.02]	0.17
Plasma HIV RNA [d]	0.38	[0.23;0.53]	<0.0001	0.26	[0.05;0.46]	0.02
CD4 cell count [e]	-0.11	[-0.18;-0.05]	0.002	-0.06	[-0.11;-0.01]	0.02
Cumulative viremia over the last 5 years [f]	1.15	[0.53;1.78]	0.0007	0.43	[-0.25;1.13]	0.20
Model 2 (Patients with undetectable HIV RNA)						
Sex (Female versus Male)	0.18	[-0.08;0.44]	0.18	0.19	[-0.07;0.46]	0.15
Age [c]	0.01	[-0.05;0.07]	0.68	0.02	[-0.04;0.07]	0.60
CD4 cell count [e]	0.00	[-0.05;0.06]	0.89	0.03	[-0.05;0.06]	0.79
Cumulative viremia over the last 5 years [f]	0.71	[0.07;1.36]	0.03	0.81	[0.15;1.47]	0.02

[a] Multivariate analysis was performed by linear regression, [b] adjusted for variables included in the model, [c] per year, [d] per $\log_{10}$ HIV RNA copies/ml of plasma, [e] per 100 cells/µl, [f] per 10000 days x $\log_{10}$ HIV RNA copies/ml of plasma.

Figure legend

Figure 1: A. The HIV DNA load in PBMCs is expressed in $\log_{10}$ copies/10^6 PBMCs and presented on the basis of current and past HAART status: HAART naive (untreated patients who had never received HAART), HAART interruption (untreated patients who had previously been on HAART), HAART HIV RNA >= 80 copies/ml (receiving HAART with detectable HIV RNA) and HAART HIV RNA < 80 copies/ml (receiving HAART with undetectable HIV RNA). Bars represent median and IQR values. B, C and D: the HIV DNA load is presented as a function of the HIV RNA level, CD4 cell counts and CD8 cell counts, respectively. Pearson's correlation coefficients for and P values are indicated.