Early and Highly Suppressive Antiretroviral Therapy Are Main Factors Associated With Low Viral Reservoir in European Perinatally HIV-Infected Children

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Background: Future strategies aiming to achieve HIV-1 remission are likely to target individuals with small reservoir size.

Setting: We retrospectively investigated factors associated with HIV-1 DNA levels in European, perinatally HIV-infected children starting antiretroviral therapy (ART) <6 months of age.

Methods: Total HIV-1 DNA was measured from 51 long-term suppressed children aged 6.3 years (median) after initial viral suppression. Factors associated with $\log_{10}$ total HIV-1 DNA were analyzed using linear regression.

Results: At ART initiation, children were aged median [IQR] 2.3 [1.2–4.1] months, CD4% 37 [24–45] %, CD8% 28 [18–36] %, $\log_{10}$ plasma viral load (VL) 5.4 [4.4–5.9] copies per milliliter. Time to viral suppression was 7.98 [4.6–19.3] months. After suppression, 13 (25%) children had suboptimal response [≥2 consecutive VL 50–400 followed by VL <50] and/or experienced periods of virological failure [≥2 consecutive VL ≥400 followed by VL <50]. Median total HIV-1 DNA was 43 [6195] copies/10^6 PBMC. Younger age at therapy initiation was associated with lower total HIV-1 DNA (adjusted coefficient [AC] 0.12 per month older, *P* = 0.0091), with a month increase in age at ART start being associated with a 13% increase in HIV DNA. Similarly, a higher proportion of time spent virally suppressed (AC 0.10 per 10% higher, *P* = 0.0022) and the absence of viral failure/suboptimal response (AC 0.34 for those with fail/suboptimal response, *P* = 0.0483) were associated with lower total HIV-1 DNA.

Conclusions: Early ART initiation and a higher proportion of time suppressed are linked with lower total HIV-1 DNA. Early ART start

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and improving adherence in perinatally HIV-1–infected children minimize the size of viral reservoir.

Key Words: HIV-1, children, reservoir, HIV DNA, early treated, viral load

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INTRODUCTION

A major obstacle to HIV remission is the persistence of virus as integrated HIV-1 DNA in long-lived cells even after many years of suppressive antiretroviral therapy (ART).

Different methodologies have been proposed to measure latent and persistent infection after initial viremia.[1] Total HIV-1 DNA in blood $CD4^+$ T cells and in peripheral blood mononuclear cells (PBMCs) are often used as a marker of the latent reservoir of HIV-1. Initiation of ART in the first year of life can minimize the size of the long-lived reservoir of HIV-1 DNA in resting memory $CD4^+$ T cells and limits the viral evolution.[2–5]

Guidelines recommend initiation of ART in all infants infected with HIV, prioritizing those younger than 1 year. Initiation of ART before postnatal peak decreases quickly the viral load (VL), reduces the period of viremia, and might favorably influence the viral reservoir in the peripheral blood and other organs.[6,7] The optimal timing for ART initiation associated with a significant reduction of the reservoir size is controversial.

All reports of HIV-1 patients with long-term remission off ART have been driven by early ART initiation, rapid viral suppression, and small HIV reservoir size after ART.[8–10] However, early ART alone is unlikely to achieve long-term, durable viral remission in the majority of patients. In a nonhuman primate model of infection, ART initiated 3 days after simian immunodeficiency virus infection did not prevent the establishment of a viral reservoir in tissues, but delayed the viral rebound when ART was withdrawn.[11] Several host factors can influence the final size of viral reservoir in a patient.[12] Timing of in utero infection, genetic differences, coinfections, time to suppression, baseline $CD4^+$ cells, baseline VL, blips, CD4:CD8 ratio, natural killer cell function, antibody response, innate immunity, and breadth and magnitude of HIV-1–specific $CD4^+$ T cells may be also associated with the viral reservoir size.[6,13,14] Better defining the influence of those factors and knowing the reservoir size of the patients are critical points to identify potential interventions aimed at inducing ART-free HIV-1 control.

Our aim in this study was to investigate the optimal timing for ART initiation and the factors associated with HIV-1 DNA in early treated European HIV-1 perinatally infected children.

This study was conducted within the frame of EPIICAL project. EPIICAL is an international consortium whose first aim is to identify profiles of viral control. These profiles will be used as end points in future proof of concept studies with novel HIV immunotherapeutic strategies.[15]

METHODS

This is a retrospective, multicenter observational survey. Data were retrospectively collected from the standardized databases of 5 European cohorts in 3 countries (see Table 1, Supplemental Digital Content, http://links.lww.com/QAI/B190).[2,4,16–18] DNA determinations were performed between 2000 and 2016 as part of clinical care in 5 of the EPIICAL European partners.[2,4,16–20] Data were gathered between September 2016 and July 2017. The study was approved by the competent ethics committees.

Eligible participants were HIV-1 perinatally infected children or adolescents who started ART in the first 6 months of life, achieved viral suppression ($\geq$2 consecutive HIV RNA $<$50 copies/mL) on ART, and had any DNA determination after viral suppression. Children were excluded if they had poor adherence to the ART during the first year of life.

The primary outcome was total HIV-1 DNA copies/10^6 PBMC. If patients had $>$1 DNA measurements, only the last DNA available data were included.

Variables investigated included age at ART start, duration of ART by HIV DNA determination, AIDS diagnosis, initial ART regimen, cohort, age at HIV DNA measurement, baseline VL, baseline immunological data (CD4% and count, CD8%, CD4/CD8 ratio, and total lymphocyte count), and postsuppression VL features (blips, spikes, suboptimal response, and failure). Variables considered a priori to be associated with HIV-1 DNA such as time to initial VL suppression and proportion of time virally suppressed were also included in the analysis.

Baseline VL defined as the closest VL measurement within 6 months before and 1 week after ART start, baseline CD4%, CD8%, CD4:CD8 ratio, lymphocyte count defined as closest measurement within 6 months before and 1 month after ART start, blips defined as single VL 50–400 copies per milliliter preceded and followed by VL $<$50, high magnitude blips (ie, spikes) defined as single VL $\geq$400 preceded and followed by VL $<$400, and suboptimal viral response defined as $\geq$2 consecutive VL 50–400 followed by VL $<$50. Virological failure was defined as $\geq$2 consecutive VL $\geq$400. The following composite variables were also considered: suboptimal viral response or virological failure, blips or spikes, spikes or suboptimal viral response, and blips or spikes or suboptimal viral response. Blips, spikes, suboptimal response, and virological failure were counted from initial VL suppression to HIV DNA measurement.

Laboratory Methods

Before 2003, standard HIV-1 RNA testing (Amplicor HIV-1 monitor; Roche Diagnostic Systems, Branchburg, NJ) was used in all laboratories, with routine lower limit of detection (LOD) of $<$400 copies per milliliter and ultrasensitive LOD of $<$50 copies per milliliter. Since 2003, participating laboratories use ultrasensitive measurements with an LOD of 20–50 copies per milliliter.

Total HIV-1 DNA

In the United Kingdom, $CD4^+$ T cells were enriched from frozen PBMC samples by negative selection (Dynabeads; Thermofisher Scientific, MA) to a purity of more than 97%. $CD4^+$ T-cell DNA was extracted (Qiagen, Hilden,

TABLE 1. Virological and Immunological Characteristics

	CHIPS/CHERUB-YC (n = 8)	CoRISpeS-CAT (n = 6)	CoRISpeS-MADRID (n = 10)	Padova (n = 12)	Rome (n = 15)	*P*†	All_Cohorts
				Median (IQR) [N]			
Age at ART start (mo)	4 (2–5) [n = 8]	2 (1–2) [n = 6]	2 (0–2) [n = 10]	2 (2–4) [n = 12]	3 (2–5) [n = 15]	0.1068	2 (1–4) [n = 51]
Age at HIV DNA measurement (years)	10 (9–13) [n = 8]	6 (4–7) [n = 6]	9 (7–11) [n = 10]	5 (4–12) [n = 12]	5 (3–13) [n = 15]	0.1669	7 (4–11) [n = 51]
Duration of ART by HIV DNA (years)	10 (9–12) [n = 8]	6 (4–7) [n = 6]	9 (6–11) [n = 10]	5 (4–11) [n = 12]	5 (3–13) [n = 15]	0.1704	7 (4–11) [n = 51]
Time to VL suppression (months)	8 (5–10) [n = 8]	7 (5–10) [n = 6]	7 (4–47) [n = 10]	14 (6–30) [n = 12]	6 (5–10) [n = 14]	0.7776	8 (5–19) [n = 50]
Proportion of time VL suppressed (%)	91 (85–95) [n = 8]	93 (84–94) [n = 6]	67 (59–82) [n = 10]	71 (39–88) [n = 12]	91 (74–95) [n = 14]	0.0735	85 (61–94) [n = 50]
HIV DNA copies/10^6 PBMC	250 (95–334) [n = 8]	21 (14–47) [n = 6]	8 (3–20) [n = 10]	26 (5–44) [n = 12]	188 (27–283) [n = 15]	0.0006	43 (6195) [n = 51]
Baseline VL (copies/ml)	224,383 (437,56–500,000) [n = 6]	650,000 (391,300–750,000) [n = 5]	21–500 (11,084–160,000) [n = 8]	750,000 (100,000–1.00e+07) [n = 11]	500,000 (21,800–500,000) [n = 9]	0.1141	247,387 (250,00–750,000) [n = 39]
Baseline log10 VL (copies/ml)	5.3 (4.6–5.7) [n = 6]	5.8 (5.6–5.9) [n = 5]	4.3 (4.0–5.2) [n = 8]	5.9 (5–7) [n = 11]	5.7 (4.3–5.7) [n = 9]	0.1141	5.4 (4.4–5.9) [n = 39]
Baseline immunological data							
CD4%	21 (15–33) [n = 7]	39 (35–47) [n = 5]	44 (44–47) [n = 5]	32 (27–45) [n = 11]	42 (31–54) [n = 13]	0.0716	37 (24–45) [n = 41]
CD8%	37 (32–44) [n = 6]	23 (16–26) [n = 5]	25 (18–28) [n = 5]	30 (18–36) [n = 9]	28 (15–33) [n = 11]	0.1903	28 (18–36) [n = 36]
CD4%/CD8% ratio	0.61 (0.48–.89) [n = 6]	1.7 (1.6–1.81) [n = 5]	1.57 (0.96–2.61) [n = 5]	0.89 (0.78–3.1) [n = 9]	1.4 (0.68–2.1) [n = 11]	0.1299	1.32 (0.73–2.2) [n = 36]
CD4 count (cells/mm^3)*	460 (195–1092) [n = 7]	—	2458 (1282–3448) [n = 4]	2195 (1450–2882) [n = 8]	2282 (503–3629) [n = 10]	0.0087	1495 (620–2885) [n = 29]
Total lymphocyte count (cells/mm^3)	2800 (2190–3000) [n = 7]	—	6061 (4160–6754) [n = 4]	7055 (5665–8840) [n = 8]	5965 (1810–7070) [n = 10]	0.0088	5600 (2800–7070) [n = 29]

Baseline VL: closest VL measurement within 6 months before and 1 week after ART start.
Baseline CD4%, CD8%, and lymphocyte count: closest measurement within 6 months before and 1 month after ART start.
Viral suppression: ≥2 consecutive VL <50 HIV RNA copies/mL.
Duration of ART by HIV DNA (years): time in years from ART start to HIV DNA measurement.
Proportion of time VL suppressed (%): duration of VL suppression from ART start to HIV DNA measurement/duration of ART by HIV DNA × 100. For calculation of duration of VL suppression, single-isolated blips or spikes were allowed and counted as time suppressed.
*CD4 count calculation: CD4% × lymphocyte count/100.
†The Kruskal–Wallis test.

Germany) and used as input DNA for droplet digital polymerase chain reaction (ddPCR; detection limits <3–4 copies/10^6 cells). Cell copy number and total HIV-1 DNA levels were quantified in triplicate using previously published assays.[4]

In Spain, $CD4^+$ T cells were purified from PBMC by negative immunomagnetic separation ($CD4^+$ T Cell Isolation Kit; Miltenyi Biotech, Auburn, CA), and lysed extracts were used to measure cell-associated total HIV-1 DNA by ddPCR with 5′LTR or Gag primers and probes, depending on the efficiency of detection in each patient (detection limits <3–4 copies/10^6 cells). DNA was calculated per 10^6 $CD4^+$ T cells and converted into 10^6 PBMC after knowing average survival PBMC under the same conditions (70%).[16,19]

In Padova, PBMCs were isolated from peripheral blood by centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient and cryopreserved in liquid nitrogen until use. Cell lysates were used to estimate cell-associated HIV-1.[21] HIV-1 DNA copies were quantified by ddPCR (QX200TM Droplet Digital TM PCR; Biorad, Bio-Rad Laboratories, Inc., Hercules, CA; detection limits 1 copy/10^5 cells), using primer pair and probe that recognizes a conserve region of the *gag* gene, and primer pair and probe that detects the housekeeping β–*actin* gene to normalize for cell equivalents.[17,21]

In Rome, total cell DNA was extracted from PBMC pellets with High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals, Germany). Total HIV-DNA was

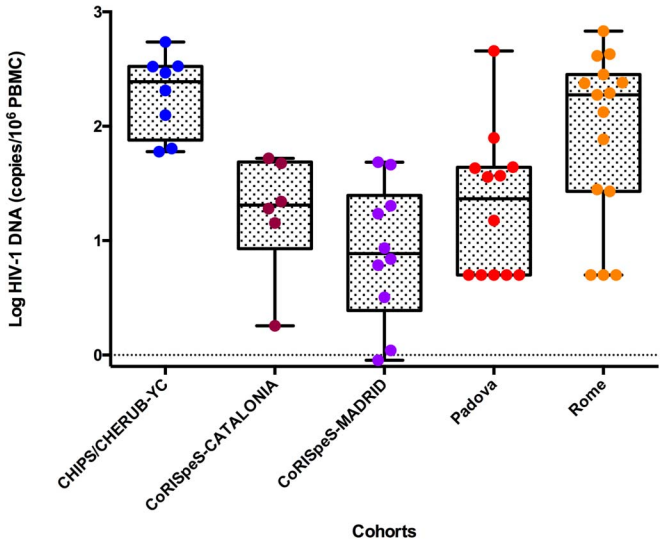

FIGURE 1. Distribution of HIV-1 DNA by cohort. Log_{10} HIV-1 DNA varies by cohort with highest levels in CHIPS and ROME (Kruskal–Wallis P value = 0.0006).

quantified by RT-PCR, using 59 nuclease assay in the long terminal repeat (LTR) region of HIV-1 (reference sequence HXB2) performed on a LightCycler v3.5 (Roche Molecular Biochemicals, IN).

Statistics

Data were analyzed using Stata software, version 14.2 (Stata Statistical Software: StataCorp LP, Release 14, College Station, TX). All patients with available DNA data from the cohorts of United Kingdom, Italy, and Spain were included.[4,16–18] HIV-1 DNA was log_{10} transformed and regressed on putative predictors. For the purpose of the analysis, if the original value was below the detection limit, the value was replaced by half the detection limit. Relating baseline VL, if original values were below lower detection limit, the value was replaced by half detection limit; if original values were over upper detection limit, they were replaced by upper limit value. For descriptive analysis, categorical variables are presented with frequency distributions (both absolute and relative). Continuous variables are summarized as median, interquartile range [IQR].

Factors associated with log_{10} total HIV-1 DNA levels were investigated using linear regression. Regression diagnostics were examined to ensure that all model assumptions were met, particularly normality of residuals further tested using the Shapiro–Wilk test.

The confounding effects of cohort and age at DNA measurement were taken into account. Variables significantly associated with log_{10} total HIV-1 DNA levels at $P < 0.10$ were included in a multivariable model, using backward stepwise elimination (exit probability $P = 0.05$) to reach the final model. Sensitivity analyses were performed excluding: (1) 5 children who experience virological failure for a period after initial suppression but suppressed for years until HIV-1 determination; and (2) 6 children who took more than 3 years to attain initial VL suppression after ART start.

To further confirm results from complete case analysis, univariable and multivariable analyses were performed using multiply imputed data. Missing data in baseline CD4%, CD8%, VL, and total lymphocyte count were multiply imputed (n = 25) using multiple imputation by chained equations.

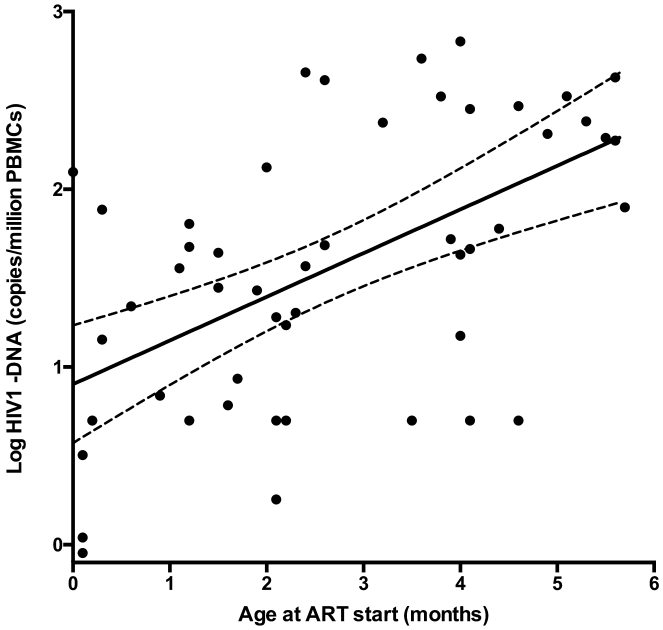

FIGURE 2. Two-way scatter plot illustrating graphically the relationship between HIV DNA and age at ART start. This plot illustrates the significant results from linear regression analysis shown in main results table.

RESULTS

In total, 51 children were included in the analysis. Fifty-five percent of the children were female, and 29% had ever had an AIDS diagnosis. Ten out of 15 (66%) patients with AIDS diagnosis had a confirmed AIDS-defining event before ART start. Baseline characteristics of patients at initiation of ART are summarized in Table 2, Supplemental Digital Content, http://links.lww.com/QAI/B190 and Table 1, and Table 3, Supplemental Digital Content, http://links.lww.com/QAI/B190. A total of 53% were from Italy, 31% from Spain, and 16% were from United Kingdom. Total HIV-1 DNA varied by cohort, with the highest levels in CHIPS/CHERUB-YC and Rome (Figs. 1 and 2). Hence, all analyses were adjusted for cohort and age at HIV-1 DNA determination.

Median [IQR] baseline VL was 247,387 [25,000–750,000] copies per milliliter. Median time to suppression was 8 [5–19] months. The participants were virally suppressed for a median of 85% (61–94) of time before DNA determination, which was 6 years [3–8] after initial viral suppression. The median [IQR] age at DNA determination was 7 [4–11] years. Median total HIV-1 DNA level was 43 [6195] copies/10^6 PBMC.

TABLE 2. Summary of VL Features After Initial Confirmed Suppression

	CHIPS		CORISPE-CAT		Madrid		Padova		Rome			Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	*P**	No.	%
Blip											0.0012		
No	1	12.5	6	100	8	80	3	25	10	67		28	55
Yes	7	87.5	0	0	2	20	9	75	5	33		23	45
Spike											0.0904		
No	7	87.5	5	83	8	80	6	50	14	93		40	78
Yes	1	12.5	1	17	2	20	6	50	1	7		11	22
Suboptimal VL response											0.2416		
No	5	62.5	6	100	7	70	9	75	14	93		41	80
Yes	3	37.5	0	0	3	30	3	25	1	7		10	20
VL failure											0.4879		
No	8	100	6	100	8	80	10	83	14	93		46	90
Yes	0	0	0	0	2	20	2	17	1	7		5	10
Composite variables													
Blip/spike											0.0068		
No	1	12.5	5	83	6	60	2	17	10	67		24	47
Yes	7	87.5	1	17	4	40	10	83	5	33		27	53
Spike/suboptimal response											0.1041		
No	5	62.5	5	83	5	50	5	42	13	87		33	65
Yes	3	37.5	1	17	5	50	7	58	2	13		18	35
Fail/suboptimal response											0.2654		
No	5	62.5	6	100	6	60	8	67	13	87		38	75
Yes	3	37.5	0	0	4	40	4	33	2	13		13	25
Blip/spike/suboptimal response											0.0046		
No	0	0	5	83	4	40	2	17	9	60		20	39
Yes	8	100	1	17	6	60	10	83	6	40		31	61

*The Kruskal–Wallis test.

After initial viral suppression, 45% of children had ≥1 blip, 22% had ≥1 spike, and 20% had suboptimal viral response. Five patients (10%) had VL failure.

When composite variables were considered, 13 (25%) children had suboptimal response and/or experienced periods of virological failure. Other composite variables are summarized in Table 2.

In multivariable analysis, lower total HIV-1 DNA was associated with younger age at ART start (adjusted coefficient 0.12; 95% CI: 0.03 to –0.21, $P = 0.0091$), with a month increase in age at ART start being associated with a 13% increase in HIV-1 DNA.

Similarly, a higher proportion of time spent virally suppressed (adjusted coefficient −0.10, 95% CI −0.17 to −0.04, per 10% higher, $P = 0.0022$) and the absence of viral failure/suboptimal response (adjusted coefficient 0.34, 95% CI: 0.00 to 0.67, for those with fail/suboptimal response, $P = 0.0483$) were associated with lower total HIV-1 DNA (Table 3). In addition, the effect of cohort remained significant with highest total HIV-1 DNA values in the cohorts from the CHIPS/CHERUB-YC (United Kingdom) and Rome. These associations remained significant after adjustment for the other factors in the multivariable model.

Although univariable analysis suggested an association between baseline immunological data (CD4, CD4:CD8 ratio, and total lymphocyte; $P = 0.05$–0.10) and also AIDS diagnosis ($P < 0.05$) and total DNA, these associations failed to reach significance after adjustment. Missing data rates in immunological data (CD4%, CD8%, and total lymphocyte) and VL ranged from 20% to 43%. Sensitivity analyses (excluding 5 children with viral failure, or 6 who took ≥3 years to suppress) as well as analyses of imputed immunological data produced similar results. Therefore, these children were not excluded. Data relating the 6 patients who took ≥3 years to suppress are provided in Table 4, Supplemental Digital Content, http://links.lww.com/QAI/B190.

DISCUSSION

In this study, we demonstrate that children who start ART earlier in life and remain suppressed without blips or treatment failure have the lowest viral reservoir, as they mature through childhood. To the best of our knowledge, this is the study with the largest number of perinatally infected children starting ART <6 months of age in Europe.

We consider that this is the ideal population to investigate immunotherapeutic strategies toward HIV remission.[22] Understanding conditions associated with different levels of HIV-1 DNA could critically help in the design of future trials.

Previous reports have shown significant differences in HIV-1 DNA measurements between groups of children

TABLE 3. Factors Associated With Lower Total HIV-1 DNA

		Univariable Model			Multivariable Model		
Factors	**N**	**% Diff**	**β (95% CI)**	***P***	**% Diff**	**β* (95% CI)**	***P*†**
Age at ART start (per mo older)	51	27.7	0.24 (0.14 to 0.35)	**0.00002**	12.7	0.12 (0.03 to 0.21)	**0.0091**
Age at HIV DNA measurement (per yr older)	51	5.3	0.05 (0.00 to 0.10)	**0.0395**	—	—	—
Duration of ART by HIV DNA (per yr higher)	51	5.0	0.05 (−0.00 to 0.10)	0.0528	—	—	—
Proportion of time suppressed (per 10% higher)†	50	−3.5	−0.04 (−0.13 to 0.06)	0.4454	−9.9	−0.10 (−0.17 to −0.04)	**0.0022**
Time to VL response (per mo longer)†	50	0.2	0.00 (−0.01 to 0.01)	0.6976	—	—	—
AIDS diagnosis	48						
Yes [Ref: No]	15	93.2	0.66 (0.20 to 1.11)	**0.0054**	—	—	—
Composite: fail/suboptimal response	51						
Yes [Ref: No]	13	55.0	0.44 (−0.05 to 0.92)	0.0758	40.1	0.34 (0.00 to 0.67)	**0.0483**
Baseline immunological data							
CD4 count (per 500 cells higher)	27	−8.7	−0.09 (−0.18 to 0.00)	0.0541	—	—	—
CD4% (per 10% higher)	41	−13.2	−0.14 (−0.29 to 0.01)	0.0625	—	—	—
CD4/CD8 ratio (per unit higher)	36	−19.0	−0.21 (−0.42 to −0.00)	**0.0479**	—	—	—
Total lymphocyte (per 500 cells higher)	27	−4.3	−0.04 (−0.09 to 0.00)	0.0678	—	—	—
Cohort [Ref: Rome]	51						
CHIPS/CHERUB-YC	8	43.1	0.36 (−0.19 to 0.90)	0.1934	29.5	0.26 (−0.17 to 0.69)	0.2326
CORISPE-CAT	6	−49.5	−0.68 (−1.29 to −0.08)	**0.0270**	−38.6	−0.49 (−0.97 to −0.01)	**0.0468**
CORISPE-MADRID	10	−64.1	−1.02 (−1.53 to −0.52)	**0.0002**	−68.3	−1.15 (−1.59 to −0.71)	**0.0000**
Padova	12	−46.2	−0.62 (−1.10 to −0.14)	**0.0130**	−59.6	−0.91 (−1.30 to −0.51)	**0.0000**

β, unadjusted regression coefficient estimates; 95% CI, confidence interval; % Diff, % difference in HIV-1 DNA: a unit change in factors investigated is associated with a Y% change in HIV-1 DNA eg, a month increase in age at ART start is associated with a 27.7% increase in HIV-1 DNA.

β*, adjusted regression coefficient estimates; *P*-value*, adjusted for the other factors included in the multivariable model.

Criteria for inclusion into the multivariable model: univariable model p-value < 0.10 or †defined a priory.

P-values in bold are those that reached statistical significance $P < 0.05$.

treated in the first month of life versus children treated late.[16,23–26] Rather than grouping patients in subsets, we have quantified the weight of each month delay in the initiation of ART after birth and the impact on the reservoir size. The results show that for each month delayed with treatment, HIV-1 DNA increases by 13% ($P = 0.009$).

Defining the interplay between timing of ART, size of reservoir, and duration of viral suppression is critical. Our study confirms the relevance of early ART initiation as well as long-term maintenance of stable viral suppression as key factors leading to lower size of viral reservoir.[27] Results suggest that for each 10% increase in proportion of time suppressed during follow-up, HIV-1 DNA decreases by 10%.

Conversely to other studies, we did not demonstrate a correlation between time to suppression and HIV-1 DNA levels.[23] A fast control is probably not enough for achieving a small reservoir. Still, this approach is currently under investigation in studies as IMPAACT 1115 (NCT02140255).

The levels of DNA in our cohort are higher than HIV-1 DNA levels found in selected children who achieved virological control before 1 year of age.[5] However, in the children who started ART in the first 2 weeks of life, the HIV-1 DNA was extraordinarily low (<1 $\log_{10}/10^6$ PBMC), similarly to the very early treated children described by Luzuriaga et al.[24]

Total HIV-1 DNA in PBMCs is not a perfect marker of reservoir size, as PCR methods can detect up to 90% defective proviruses.[28,29] In one report, of the 9 viral outgrowth assays performed for early treated youth, only 1 of 20 replicates tested positive for replication-competent virus.[24] Although it is tempting to hypothesize that children with <10 copies/10^6 PBMC may have virtually no replication competent virus, replication-competent HIV-1 can be recovered from individuals with HIV-1 DNA levels below 2 copies/10^6 PBMC.[30] Rebound of viremia may follow after cessation of ART despite very low levels of replication-competent HIV-1, suggesting the stochastic nature of virus rebound.[1,16] Further assays as measurements after rounds of T-cell activations, sequencing, and measurement of reservoir in tissues should be tested in the future.[31]

Residual HIV-1 replication has been suggested to contribute to the maintenance of the HIV-1 reservoir.[24] According to our results, brief intermittent viremia (ie, blips and spikes) did not have a long-term impact; however, prolonged low-grade viremia and/or virological failure led to higher levels of HIV-1 DNA.

Power was also insufficient to confirm an association between HIV-1 DNA and baseline immunological data. However, CD4:CD8 ratio ($P = 0.0479$ in the univariate model) seemed a potential predictor for a higher reservoir. Of note, CD4:CD8 has also been associated with T-cell activation, senescence, and exhaustion.[32]

In our study, the effect of site remained significant with highest HIV-1 DNA values noted in the United Kingdom and Rome. Also, significant cohort differences were found for AIDS diagnosis, $CD4^+$ counts, total lymphocyte counts, and blips. However, there were no significant differences for year of ART initiation. UK children presented with more AIDS diagnosis, lower baseline CD4, and total lymphocyte counts.

Median of ART start in United Kingdom and Rome was 1–2 months later than in other cohorts (Table 1). At that time point, treatment policy in United Kingdom was to initiate ART only when children were symptomatic. Although 1–2 months of delay may seem a small amount of time, first 3 months of life may be key for the immunological response to HIV.[7] Bearing in mind that each month of delay in initiating ART implies a 13% increase in the size of the reservoir, it is normal that in the UK cohort the reservoir is at least 25% higher than in Italy and Spain.

Time from ART to HIV-1 DNA measurement was longer in the United Kingdom than in other cohorts, because of DNA measurement at older age. This is why proportion of time spent suppressed was deemed as a more accurate indicator of effective ART, accounting for duration of VL suppression and duration of ART by HIV DNA measurement.

Numbers starting nonnucleoside reverse transcriptase inhibitor–based or protease inhibitor–based regimen by cohort are low for comparison. In general, UK children had higher HIV DNA, regardless of initial ART regimen. We were aware of a significant cohort effect, and adjustment for cohort was done in the final multivariable analysis.

This study had some operational difficulties, as determinations were performed by different laboratories. Limitations of this study include different PCR techniques and cutoff limits used for VL and DNA, which may have had an influence in the homogeneity of data and results. Both ddPCR for HIV-1 in PBMCs and ddPCR for HIV-1 in $CD4^+$ T cells detect the same viral species (replication-competent and replication-defective). However, the former analyzes not only $CD4^+$ T cells but also monocytes/macrophages. An adjustment from UK and Spain data was necessary for comparison, taking into account the number of viable PBMC of the analyzed samples. In comparative studies, the levels of HIV-1 DNA in unfractionated PBMCs and in purified resting $CD4^+$ T cells showed a strong correlation ($r = 0.78$, $P = 0.0004$).[1] In any case, there is a need for standardization of assays used to measure the reservoir, especially if these are to be used in future clinical trials.

We had no information about additional host factors that may influence the reservoir size. However, cross-sectional and prospective ongoing studies conducted within the EPIICAL consortium will address other factors.

The small sample size limited the power to identify other factors associated with total HIV-1 DNA level after viral suppression. Longitudinal data to assess reservoir decay, and complete serology data were also missing.

Despite these limitations, this is one of the studies with the highest number of cases of early treated HIV-infected children measuring the viral reservoir and evaluating the associated factors, so far.

In conclusion, a long-term lower total HIV-1 DNA was found to be associated with starting ART earlier and with spending a higher proportion of time suppressed. This study provides further evidence to support current guidelines that recommend treatment with ART as soon as possible in perinatally infected children.

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