

Pediatric Viral Human Immunodeficiency Virus Type 1 RNA Levels, Timing of Infection, and Disease Progression in African HIV-1-Infected Children

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ABSTRACT. *Objective.* To describe plasma human immunodeficiency virus type 1 (HIV-1) RNA levels in African HIV-1-infected children in relation to the timing of infection and disease progression.

Methods. A retrospective cohort study was conducted of 80 children who were born to HIV-1-positive mothers and clinically followed from birth to 18 months of age in the ANRS 049 Ditrane project, Abidjan, Côte d'Ivoire (West Africa). The diagnosis and timing of pediatric HIV-1 infection were determined prospectively according to HIV-1 DNA polymerase chain reaction results. A total of 364 HIV-1 RNA viral load (VL) measurements were assessed retrospectively. Kaplan-Meier analyses and proportional hazards models were used to evaluate the prognostic value of pediatric VL and covariates for HIV disease progression or death.

Results. Mean initial positive VL was significantly lower among children who were infected in utero (4.94 log₁₀/mL, *n* = 12) than in children who were infected later (5.6–6.1 log₁₀/mL, *n* = 68). In the first 6 months after diagnosis, HIV-1 RNA levels peaked (≥6 log₁₀/mL), regardless of timing of infection. Then, a slow decline (overall slope, –0.076 log₁₀ copies/mL/mo) was observed until 18 months of age. A 1 log₁₀ higher value of the pediatric peak VL (risk ratio [RR]: 1.85; 95% confidence interval [CI]: 1.0–3.44) and of the maternal VL at delivery (RR: 1.90; CI: 1.16–3.12) were independently associated with an increased risk of rapid progression to acquired immune deficiency syndrome (AIDS) or death at 18 months of life (23 AIDS diagnoses and 31 deaths). Disease progression or death was more rapid for girls than for boys (RR: 2.26; CI: 1.39–4.96).

Conclusions. In Africa, pediatric HIV-1 RNA levels are very close to those described in industrialized countries and seem to be predictive of AIDS stage or death, as in industrialized countries. With antiretroviral therapy becoming more widely available, the early identification and monitoring of pediatric HIV disease remains of paramount

importance in Africa. *Pediatrics* 2003;112:e289–e297. URL: <http://www.pediatrics.org/cgi/content/full/112/4/e289>; HIV-1 infection, African children, viral load, disease progression.

ABBREVIATIONS. HIV-1, human immunodeficiency virus type 1; ART, antiretroviral therapy; ARV, antiretroviral; PMTCT, prevention of mother-to-child transmission; ZDV, zidovudine; BC, benzalkonium chloride; PCR, polymerase chain reaction; CDC, Centers for Disease Control and Prevention; bDNA, branched DNA; SD, standard deviation; VL, viral load; RR, risk ratio; CI, confidence interval.

In Europe and North America, where perinatal human immunodeficiency virus type 1 (HIV-1) transmission rates are as low as 1%,¹ many advances in the knowledge and management of pediatric HIV-1 infection have been made. A bimodal clinical evolution of disease was identified more than a decade ago in vertically HIV-1-infected children who lived in industrialized countries, with children who were infected in utero having a more acute and rapidly evolving HIV disease than those who were infected later.² Besides timing of transmission,³ maternal characteristics (eg, health status, gestational age),⁴ and viral phenotypes,⁵ children's age-related CD4⁺ T-cell count has been identified as a major independent and early predictor of disease progression or death in children.⁶ Since assays that quantify plasma HIV-1 RNA copy number have become available, HIV-1 dynamics have been well characterized during primary infection in adults^{7–9} and in perinatally infected children.^{10–12} Numerous studies performed in adults have demonstrated that HIV-1 RNA levels are independent predictors of disease progression and have resulted in clinical guidelines.^{13–15} In contrast, pediatric studies are scarce and antiretroviral therapy (ART) guidelines remain broad. In the Women and Infants Transmission Study cohort, Shearer et al¹² reported that the median HIV-1 RNA levels over the first 2 months of life in infected children were predictive of an increased rate of disease progression. Mofenson et al¹⁶ described an independent association of CD4 lymphocyte percentage and RNA level with mortality risk in a cohort of infected children who were enrolled in an intravenous immunoglobulin prophylaxis trial. Palumbo et al¹⁷ documented a strong association

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between disease progression and baseline RNA levels across a wide range of ages in infants who were enrolled in the ACTG 152 trial. Most of these pediatric studies,^{11,12,16–19} except a few,^{20,21} included a significant proportion of children who were under antiretroviral (ARV) treatment.

All of these advances have had little impact in sub-Saharan Africa, where >90% of the 3 million children who are infected with HIV are currently living.²² The burden of pediatric infection in Africa is likely to increase in the future despite encouraging initiatives focusing on the use of abbreviated ARV regimens for the prevention of mother-to-child transmission (PMTCT).²³ The HIV-1 dynamics and its prognostic value in African children have not been investigated except in Malawi.^{24,25} The natural history of pediatric HIV-1 disease requires additional documentation according to the route of infection (in utero, peripartum, and postpartum via breastfeeding). In addition, the relationship between HIV RNA levels and pediatric disease progression needs to be studied in Africa, where environmental exposure and nutritional factors, as well as HIV genotypes, are different from those observed in industrialized countries. Thus, additional guidance could be given on the timing of initiation of ARV treatment in HIV-infected children who live in Africa.

This report focuses on the HIV-1 plasma RNA copy number obtained from birth to the first 18 months of life in 80 HIV-infected children who were born to HIV-infected women who live in Abidjan, Côte d'Ivoire (West Africa). No ARV treatment was available in the country when this study was performed. Patterns of HIV-1 replication were described in relation to the estimated time of infection and the rate of disease progression or death.

METHODS

Child Population

The study population consisted of HIV-1-infected children who were born to 427 pregnant women aged 18 years or older, with confirmed HIV-1 infection, and enrolled in the ANRS 049 Ditrane project (Côte d'Ivoire, West Africa), which consisted of 2 clinical trials and an open zidovudine (ZDV) cohort. Details about enrollment and follow-up procedures in the project have been described elsewhere.^{26–28} Briefly, the project was conducted in Abidjan, the economic capital of Côte d'Ivoire (3 million inhabitants). The study population was recruited from the Yopougon district, which is the most densely populated, with 500 000 inhabitants. In this district, a university hospital and a network of public clinics offer prenatal, obstetric, and pediatric care. All of the children were from families with limited resources, and approximately 45% came from very poor families (income <1€ a day). First, between September 1995 and February 1998, the double-blind, placebo-controlled ANRS 049a trial assessed the efficacy of a short course of ZDV given to 250 mothers for PMTCT.²⁶ Briefly, women were randomized at 36 to 38 weeks' gestation to receive ZDV or a matching placebo: 250/300 mg twice daily until the beginning of labor, then a single oral dose of 500/600 mg until delivery, then a 7-day postpartum treatment of 500/600 mg/d. Second, from November 1996 to April 1997, children were recruited from 40 women who were participating in a double-blind, placebo-controlled trial using benzalkonium chloride (BC) vaginal cleansing for PMTCT (ANRS 049b trial).²⁷ Women self-administered a daily vaginal suppository of 1% BC or matched placebo from 36 weeks of pregnancy and a single intrapartum dose. The neonate was bathed with 1% BC solution or placebo within 30 minutes after birth. Third, between March 1998 and November 1998, children were born to 137 mothers who were enrolled in an open-label

ZDV cohort.²⁸ This cohort was initiated when the results of the placebo-controlled trial conducted in nonbreastfeeding women in Thailand showing efficacy of a short ZDV regimen were made public.²⁹ Women received the ZDV regimen used during the ANRS 049a trial. In all cases, enrolled women gave informed consent. The 2 trials and the open cohort were approved by the national Ethics Committees of Abidjan, Côte d'Ivoire, and Bordeaux, France. No ZDV prophylaxis was given to the neonates. All but 14 infants were breastfed by their mothers. Median breastfeeding duration was 210 days (range: 0–540 days).

Sample Processing and Definitions

Whole blood was routinely collected by venipuncture (1–2 mL of EDTA-anticoagulated peripheral blood) at 8 predefined time points: days 1 to 2 or 8, 45, 90, and 180, and then every 3 months until 18 months of age. All specimens were shipped to the local laboratory (CeDReS, Centre de Diagnostic et de Recherche sur le SIDA, Abidjan) and processed within 6 hours. Peripheral blood mononuclear cells, separated from plasma using Ficoll-Hypaque, were stored at –80°C until tested for HIV-1 DNA polymerase chain reaction (PCR). HIV-1 RNA testing was subsequently performed on frozen plasma.

In the present study, according to international standards,^{30,31} the diagnosis of HIV-1 infection for each infant was based on a single positive HIV-1 DNA PCR result obtained at any age. HIV serology was also assessed in liveborn children who survived at 15 to 18 months of follow-up to validate HIV infection status.

Four categories were considered with regard to the timing of infection. In utero infection was defined as a positive HIV-1 DNA PCR within 48 hours of life.³² Children who exhibited a positive HIV-1 DNA PCR during the first 3 weeks of life but without a PCR result available during the first 48 hours were assumed to have been infected in utero/peripartum. In this category, the median time for a positive PCR result was 7 days (range: 3–17 days). Peripartum/early postnatal infection was defined as 1 positive HIV-1 DNA PCR at day 45 but negative before. Late postnatal infection through breastfeeding was defined as a negative HIV-1 DNA PCR at day 45 but positive on a subsequent specimen.

From birth, morbidity and mortality information was collected prospectively at each visit until 18 months of age. Rapid progression of disease was defined as a class C clinical event, according to the Centers for Disease Control and Prevention (CDC) 1994 revised classification system for pediatric HIV infection³³ or death by 18 months of age.

Laboratory Assays

In children, a qualitative nested homemade HIV-1 DNA PCR assay was performed with 3 pairs of specific primers (Eurogentec, Herstal, Belgium) in the *gag* (GAG881–882/SK38–39) and the *pol* (HIPOL42–45/43–44 and POL001–004/002–003) regions of the HIV-1 genome, as described elsewhere.²⁶ Briefly, sensitivity of this amplification was validated for each run by using calibrated DNA from 8E5 cells as positive controls with a detection level of 5 copies of proviral DNA per 10⁶ cells. CeDReS participated in interlaboratory quality control instituted with the laboratory of virology of Necker Hospital, Paris, France. All specimens tested by these 2 laboratories gave concordant results.

Plasma HIV-1 RNA copy number was subsequently measured using a third-generation branched DNA (bDNA) kit (Quantiplex HIV-1 RNA 3.0 assay; Chiron Diagnostics Corp, East Walpole, MA). This RNA tool was chosen because of its ability to detect accurately non-B HIV-1 genotypes.³⁴ It gave extremely concordant results in this pediatric population with the above-mentioned HIV-1 DNA PCR designed to allow a reliable amplification of both B and non-B subtypes.³⁵ This property is particularly suitable in Côte d'Ivoire, where the CRF02 recombinant form AG is predominant.³⁶ The use of the bDNA assay in a pediatric context was possible using 0.2-mL specimens and correcting the threshold of the assay to 250 (instead of 50) copies/mL. Experimentally, this approach does not affect the quantitative results of the bDNA assay.³⁷ The specimens with results above the quantitation upper limit (500 000 copies/mL) were retested after a 1:10 (or 1:20) dilution. Measurements of HIV-1 RNA copy number by bDNA were also performed on samples collected in women at delivery. HIV serology on serum samples collected in children at 15 to 18 months of age and in pregnant women at inclusion and the CD4⁺

T-cell count (using FACScan flow cytometry) among women at delivery were done as previously published.²⁶

Statistical Analysis

Log₁₀ transformations of the HIV-1 RNA copy number were used for analysis. Data are presented as mean titers and their standard deviation (SD). The 4 categories previously defined for the timing of infection were compared by using the Wilcoxon rank sum test for continuous variables (maternal CD4⁺ T-cell count at delivery, maternal HIV-1 RNA viral load [VL] at delivery, gestational age, and birth weight) and the χ^2 test for categorical variables (sex and maternal treatment arm). Comparisons of plasma HIV-1 RNA VL between groups were also made with the Mann-Whitney-Wilcoxon and the *t* tests. The diagnostic VL was defined as the first positive HIV-1 RNA VL obtained in each infected child. The peak VL was considered as the maximal HIV-1 RNA value obtained during the first 6 months after the diagnosis of infection. Repeated VL values allowed for definition of the slope after the peak value. The mean slopes were estimated using a mixed model for longitudinal data with time, infection group, and interaction between time and infection group as fixed effects, and intercept and time as random effects.³⁸ Probabilities of 18-month disease-free progression and survival were estimated using the Kaplan-Meier method, with *P* values calculated by the log-rank test. Cox proportional hazards regression models were used to assess the prognostic value of HIV-1 RNA. Multivariate Cox analyses were performed using descending stepwise procedures. All variables that verified the hypothesis for hazard proportional model and associated, in univariate analysis, with the event with a significance level <.25 were included in the stepwise procedure. The variables that were less significant were dropped one by one checking for confounding. The final multivariate model contains the variables with a significant level <.05 and others forced in the model to avoid confounding. All statistical analyses were done with SAS version 8.02 software (SAS Institute, Cary, NC).

RESULTS

Description of the Study Population

Of the 427 seropositive mothers, 85 of 418 liveborn children were diagnosed as having HIV-1 infection (53 from the 049a trial, 5 from the 049b trial, and 27 from the ZDV cohort). Five children were excluded from this study for the following reasons: plasma specimens not available at any age (*n* = 3), twin birth (*n* = 1), and negative bDNA/positive DNA PCR pattern at month 9 and positive HIV serology at month 18 (*n* = 1). Overall, from 80 singleton children (including 45 girls and 46 children who were born to mothers who received ZDV), we were able to measure plasma HIV-1 RNA VLs in 364 samples (90 negative and 274 positive samples; average: 4.5 samples per child; range: 1–8). HIV-1 DNA PCR and bDNA assay agreed in all specimens except 1, which was excluded from the analysis. Most specimens (78%) were collected before 12 months of age.

Twenty-three children (28.7%) progressed to CDC stage C, with the following nonexclusive AIDS-defining clinical signs or symptoms: weight loss (*n* = 12), marasmus (*n* = 8), failure to thrive (*n* = 5), severe pneumonia (*n* = 3), and chronic diarrhea (*n* = 3); of these, 13 children died before 18 months. For the 10 remaining children, 4 died after 18 months, 3 were lost to follow-up after 18 months, and 3 were included in another ANRS program since October 2000. They are alive in 2003, and 2 of them are under ART since May 2002. Thirty-one other children died without reaching CDC stage C (17 at stage B, 1 at stage A, and 13 asymptomatic for HIV infection). The most common causes of death among these 31 chil-

dren were pneumonia (*n* = 14) and diarrhea (*n* = 5). Seven of them (including 4 at stage N) died without any documented cause. Overall, 54 (67.5%) of the 80 children thus were considered as rapid progressors as they progressed to CDC stage C or died. Median age of developing AIDS or at death was 9.0 months.

Plasma HIV-1 RNA VL in Children

Influence of the Timing of Infection

We observed a wide range of plasma HIV-1 RNA copy numbers in the 80 infected children at all time points, from <250 to 19 000 000 copies/mL (Fig 1). Twelve (15%), 16 (20%), 32 (40%), and 20 (25%) children were defined as infected in utero, in utero/peripartum, peripartum/early postnatally, and late postnatally, respectively. There were no significant differences between the timing of infection and any variable evaluated, including sex, maternal treatment group, maternal CD4⁺ T-cell count at delivery, maternal HIV-1 RNA VL, gestational age, and birth weight (data not shown).

HIV-1 RNA dynamics in children who were infected in utero or in utero/peripartum (Fig 1a and b) showed a rapid rise until month 3 and day 45, respectively. Then, a slow decline was observed over time. For children who were infected peripartum/early postnatally (Fig 1c), HIV-1 RNA VL was below the cutoff level in the first sample around birth, rose rapidly to 6 log₁₀ copies/mL at day 45 and month 3, gradually declined by 12 months of age, and then stabilized. For children who were infected late postnatally (Fig 1d), the HIV-1 RNA pattern over time should be considered with caution, as the window of acquisition of infection was large (including 7 children who were infected between day 45 and month 3, 5 children who were infected between months 3 and 6, and 5 children who were infected between months 6 and 9).

Overall, because the highest HIV-1 RNA levels were always observed in the first 6 months after the diagnosis of infection, followed by a slow decline over 18 months of age, we focused on 4 summary measures to describe HIV-1 RNA VL pattern according to the timing of infection: the diagnostic VL (first positive VL above the threshold defining the diagnosis and the timing of HIV-1 infection; *n* = 80), the peak VL (highest value obtained in the first 6 months after the diagnosis of infection; *n* = 80), and the slope of the decline obtained thereafter (*n* = 47). As shown in Table 1, the mean diagnostic VL in children who were infected in utero was significantly lower (mean: 4.94 log₁₀ copies/mL at days 1–2) than that observed for children who were infected late postnatally (5.88 log₁₀ copies/mL; *P* < .001). Also, the in utero mean diagnostic VL was significantly lower than those obtained in the in utero/peripartum group (5.58 log₁₀ copies/mL; *P* = .037) and in the peripartum/early postnatal group (6.09 log₁₀ copies/mL; *P* < .001).

In contrast, once infection was established, the median peak values were not statistically different in the 4 groups and were always close to 6 log₁₀ copies/mL. Thus, the difference between the diagnostic and

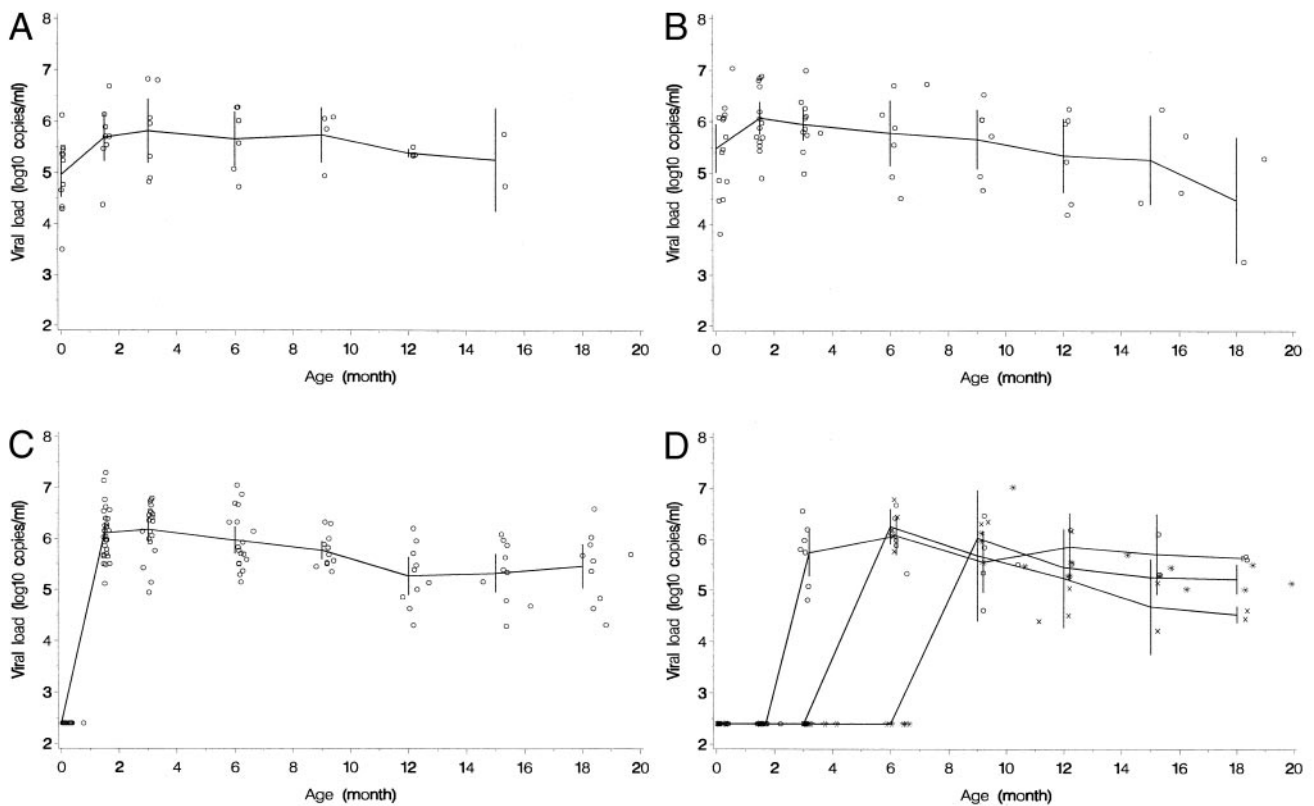


Fig 1. Plasma HIV-1 RNA VL dynamics in 80 African children who were infected with HIV-1, according to the timing of infection and age. The solid line connects the mean values of the individual data points. The vertical bars represent the 95% CIs of the means. For the definition of timing of infection, see the Methods section. Three children who were infected late postnatally were not included in d because they were contaminated in a larger window period (1 between day 45 and month 6, 1 between months 3 and 9, and 1 between months 6 and 12). In d, o indicates children HIV-negative at day 45, then positive at month 3; x, children HIV-negative at month 3, then positive at month 6; and *, children HIV-negative at month 6, then positive at month 9.

TABLE 1. Mean Plasma HIV-1 RNA VL in 80 African Children Infected With HIV-1

Timing of Infection	Diagnostic HIV-1 RNA VL*				Peak HIV-1 RNA VL†		Slope After the Peak‡		
	N	Mean Age (Days [SD])	Mean Value (Log ₁₀ Copies/mL [SD])	P§	Mean Value (Log ₁₀ Copies/mL [SD])	P§	N	Estimated Mean (Log ₁₀ Copies/mL [95% CI])	P
In utero	12	1.4 (0.65)	4.94 (0.70)	<.001	5.96 (0.70)	.77	6	-0.052 (-0.12 to 0.02)	.20
In utero/peripartum	16	8.2 (5.62)	5.58 (0.90)	.40	6.31 (0.59)	.22	10	-0.099 (-0.14 to -0.05)	.83
Peripartum/early postnatal	32	47.2 (6.91)	6.09 (1.48)	.25	6.27 (0.53)	.26	19	-0.050 (-0.08 to -0.02)	.05
Late postnatal	20	196.4 (98.3)	5.88 (0.61)	Ref.	6.07 (0.57)	Ref.	12	-0.106 (-0.15 to -0.06)	Ref.
Overall	80	69.8 (90.0)	5.76 (0.74)		6.18 (0.58)		47	-0.076 (-0.10 to -0.05)	

* The diagnostic VL was the first positive HIV-1 RNA VL obtained for each infected child.

† The peak HIV-1 RNA VL was the highest number of HIV-1 RNA copies measured during the first 6 months after the diagnosis of infection.

‡ The slope was the change in log₁₀ value per month after the peak value estimated in a mixed model.

§ By the Mann-Whitney-Wilcoxon test.

|| By *t* test in a mixed model.³⁸

peak VLs was limited (<0.3 log₁₀ copies/mL) for children who were infected peripartum/early postnatally or infected late postnatally, whereas this difference reached 1 log₁₀ copies/mL for children who were infected in utero. Finally, after the peak of viremia, the slopes of the decrease ranged from -0.050 (peripartum/early postnatal group) to -0.106 (late postnatal group) log₁₀ copies/mL/mo.

Other Factors Influencing HIV-1 RNA VL Patterns

The linear regression coefficients between HIV-1 RNA levels and other covariates of interest are

shown in Table 2. As the pediatric diagnostic HIV-1 RNA VL was significantly related to the timing of infection (see above), the corresponding coefficients were adjusted to this variable. No linear trend was observed between virus levels and the infant's sex, maternal treatment arm, gestational age, and birth weight. Maternal CD4⁺ T-cell count at delivery was inversely related with children HIV-1 RNA copy number obtained during the pediatric peak viremia (coef. = -0.07, *P* = .006; *n* = 78). Maternal VL at delivery was positively related with the pediatric diagnostic VL (coef. = 0.27, *P* = .02; *n* = 68).

TABLE 2. Linear Regression Coefficients of Pediatric Diagnostic and Peak HIV-1 RNA VLs by Sex, Maternal Treatment Arm, Gestational Age, Birth Weight, and Maternal CD4 Cell Count and VL

	N	Diagnostic HIV-1 RNA VL		Peak HIV-1 RNA VL	
		Coefficient* (95% CI)	P	Coefficient† (95% CI)	P
Sex					
Girls	45	-0.20 (-0.49 to 0.09)	.18	0.208 (-0.05 to 0.47)	.11
Boys	35	Ref.		Ref.	
Maternal treatment arm					
ZVD	46	-0.103 (-0.40 to 0.19)	.49	0.052 (-0.22 to 0.31)	.69
Placebo or BC	34	Ref.		Ref.	
Gestational age for a 1-d increase	60	-0.075 (-0.15 to 0.03)	.19	-0.038 (-0.13 to 0.05)	.39
Birth weight for a 100-g increase	78	-0.012 (-0.04 to 0.02)	.50	-0.014 (-0.04 to 0.02)	.33
Maternal CD4 ⁺ T-cell count at delivery for a 100-cell/mm ³ increase	78	-0.05 (-0.11 to 0.01)	.09	-0.07 (-0.12 to 0.02)	.006
Maternal HIV-1 RNA VL at delivery for a 1-log ₁₀ increase	68	-0.27 (0.05 to 0.50)	.02	0.14 (-0.07 to 0.35)	.19

* Adjusted and † unadjusted for the timing of infection as defined in Table 1.

HIV-1 RNA VL and Disease Progression

Using the Kaplan-Meier analysis, we did not identify a significant difference in pediatric disease progression according to the diagnostic VL, given the interaction between this virologic variable with the timing of infection, and the limited sample size in each group defining the timing of infection (data not shown). In contrast, by using the peak VL, which was independent of the timing of infection, children with peak HIV-1 RNA levels more than or equal to the median value (6.2 log₁₀ copies/mL) progressed to AIDS or died significantly faster than children with peak HIV-1 RNA levels below the median value ($P = .03$; Fig 2).

For further examining the relationship between pediatric HIV-1 RNA values and the risk of progressing to AIDS or death while controlling for potential confounding covariates, Cox proportional modeling analysis was performed. The univariate proportional hazards model, which compared the crude differences between children who progressed to AIDS or died and those who did not, are shown in Table 3. A 1-log₁₀ increase in pediatric HIV-1 RNA peak copy number was associated with the risk of developing AIDS or of progressing to death over the 18 months

of follow-up (risk ratio [RR]: 1.96; 95% confidence interval [CI]: 1.1–3.5), as well as being a girl (RR: 2.30; CI: 1.2–4.3). Neither pediatric diagnostic VL nor timing of infection, gestational age, maternal treatment, and birth weight were associated with the risk of progression to AIDS or death. A 100-cell/mm³ increase in maternal CD4⁺ cell count at delivery tended to protect ($P = .07$) against developing pediatric AIDS or of progressing to death, whereas the risk ratio was almost twice as high for a 1-log₁₀ increase in maternal HIV-1 RNA copy number at delivery. In the multivariate Cox models, pediatric HIV-1 RNA level at the time of diagnosis and the peak value were considered separately (Table 4). When assessing the contribution of the pediatric diagnostic VL, the timing of infection was a forced modeling variable because of its strong interaction. The adjusted RR of progression to AIDS or death for each 1-log₁₀ increment in pediatric peak VL was of borderline significance (RR: 1.85; CI: 1.0–3.4; $P = .05$) in contrast to the pediatric initial positive VL, which did not contribute any more to the prediction ($P = .13$). In both models, disease progression or death was more rapid for children who were born to mothers who exhibited a high VL at delivery and for girls

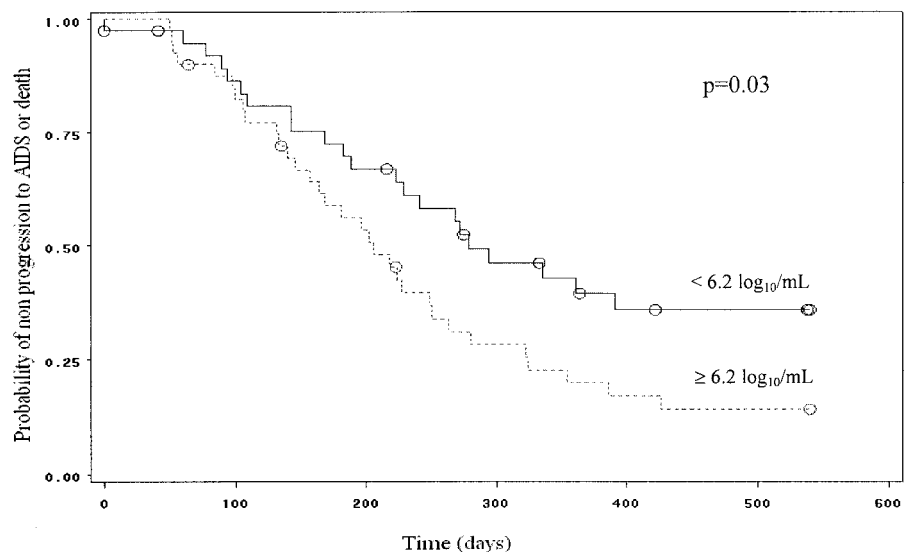


Fig 2. The Kaplan-Meier analysis shows proportion of infected children who did not progress to AIDS or death, according to the peak VL during the first 6 months after diagnosis. The median value of 6.2 log₁₀ HIV-1 RNA copies/mL was used as the threshold for predicting the occurrence of CDC stage C or death up to 18 months of age.

TABLE 3. Univariate Cox Proportional Hazards Model for the Risk of Progression to AIDS or Death in HIV-1-Infected Children in the ANRS 049 Ditrane Project

	N	RR (95% CI)	P
Child variables			
HIV-1 RNA VL			
Diagnostic VL (for a 1 log ₁₀ increase)	68	1.17 (0.78–1.74)	.45
Peak VL (for a 1 log ₁₀ increase)	68	1.96 (1.10–3.48)	.02
Timing of infection			
In utero	11	1.29 (0.52–3.18)	.58
In utero/peripartum	13	1.15 (0.48–2.78)	.76
Peripartum/early postnatal	26	1.07 (0.49–2.38)	.86
Late postnatal	18	Ref.	
Sex			
Girls	39	2.30 (1.23–4.30)	.009
Boys	29	Ref.	
Birth weight (for a 100-g increase)	67	0.96 (0.91–1.02)	.17
Maternal variables			
Treatment arm			
ZDV	38	1.25 (0.69–2.27)	.45
Placebo or BC	30	Ref.	
Gestational age (for a 1-d increase)	52	1.00 (0.84–1.21)	.95
CD4 ⁺ T-cell count at delivery (for a 100-cell/mm ³ increase)	68	0.89 (0.78–1.01)	.07
HIV-1 RNA VL at delivery (for a 1-log ₁₀ increase)	68	1.85 (1.12–3.05)	.02

TABLE 4. Final Multivariate Cox Proportional Hazards Models* for the Risk of Progression to AIDS or Death in HIV-1-Infected Children Included in the ANRS 049 DITRAME Project

	RR (95% CI)	P
Model with children diagnostic HIV-1 RNA VL		
Children diagnostic VL (for a 1-log ₁₀ increase)	1.49 (0.89–2.49)	.13
Timing of infection		
In utero	1.05 (0.34–3.23)	.93
In utero/peripartum	0.98 (0.39–2.45)	.97
Peripartum/early postnatal	0.67 (0.29–1.56)	.36
Late postnatal	Ref.	
Sex		
Girls	3.17 (1.61–6.24)	<.001
Boys	Ref.	
Maternal VL at delivery (for a 1-log ₁₀ increase)	2.07 (1.21–3.55)	.008
CD4 cell count at delivery (for a 100-cell/mm ³ increase)	0.99 (0.87–1.13)	.88
Birth weight (for a 100-g increase)	0.98 (0.91–1.06)	.67
Model with children peak HIV-1 RNA VL		
Children peak VL (for a 1 log ₁₀ increase)	1.85 (1.00–3.44)	.05
Sex		
Girls	2.26 (1.39–4.96)	.003
Boys	Ref.	
Maternal VL at delivery (for a 1-log ₁₀ increase)	1.90 (1.16–3.12)	.01
CD4 cell count at delivery (for a 100-cell/mm ³ increase)	0.99 (0.86–1.13)	.86
Birth weight (for a 100-g increase)	1.00 (0.94–1.07)	.96

* Stepwise descending procedure.

as compared with boys. Maternal CD4⁺ cell count at delivery was no longer associated with the risk of progression to pediatric AIDS or death.

DISCUSSION

This study provides an in-depth picture of plasma HIV-1 RNA patterns, stratified on the timing of infection, in a cohort of untreated African children who were followed from birth to 18 months of age and mostly infected with the HIV-1 CRF02 recombinant form AG, which is the predominant clade in Côte d'Ivoire.³⁶ Despite the vast differences between sub-Saharan Africa and industrialized countries (in terms of viral, immunologic, environmental, and nutritional factors, and route of exposure including the additional risk linked to breastfeeding and access to ARV therapy), our findings corroborate and extend in a striking manner studies performed previously in

the developed world^{11,12,16,17} that observed a unique pattern of viral replication in infected children. In adults, during the period of primary infection, HIV-1 RNA copy number initially rises to high peak levels, rapidly followed by a 2- to 3-log₁₀ decrease until reaching a steady-state plateau (the virologic “set-point”) within approximately 6 to 9 months after the acquisition of HIV infection.^{15,39} In contrast, the initial burst of viremia in children can reach higher values (>10⁶ copies/mL). In addition, RNA copy number slowly declines even without treatment during the first years after birth and persists at higher levels than those observed in most infected adults. This slow decline in the pediatric plasma HIV-1 RNA VLs probably reflects the lower efficiency of the immature immune system, which has difficulties for containing the viral replication and possibly a greater number of HIV-susceptible cells. This spon-

taneous decline, described by others, leaves uncertainty as to whether age-related changes in HIV-1 RNA levels should be taken into account when using VL measures in young children to evaluate ARV drug efficacy. Primary HIV-1 infection in children may also be influenced by the timing of infection. Indeed, in our study, most children who were infected in utero had relatively low levels of HIV-1 RNA at birth, as compared with initial positive HIV-1 RNA VL obtained in children who were infected later. This finding is consistent with previous studies conducted in industrialized countries (where a proportion of infants and their mothers during pregnancy received ARV treatment)^{11,12} and in Africa as well. For instance, among 135 untreated children from Malawi, Biggar et al²⁴ obtained a median level in 24 cord blood-positive samples of 4.89 log₁₀ copies/mL, significantly lower than levels in the first positive samples of 52 cord blood-negative perinatally infected children (median: 5.55 log₁₀ copies/mL). The generally low HIV-1 RNA levels observed at birth among children who were infected in utero suggest either that infection in utero occurred close to the time of birth or that virus replication was inhibited in the fetus by maternal or placental factors before delivery. One cannot exclude, however, the possibility of early in utero virus transmission and replication in privileged sites, such as the fetus brain or gastrointestinal tract.

Few studies have investigated the prognostic ability of the HIV-1 RNA VL by controlling the use of ARV therapy in children. In industrialized countries, Abrams et al,²¹ analyzing data from 89 HIV-1-infected children who were not exposed to ZDV prophylaxis, showed that HIV-1 RNA VL up to 18 months was strongly associated with HIV disease progression. In Africa, one study of 155 untreated children older than 1 year from Malawi²⁵ obtained a more powerful predictive value for disease progression by the combining plasma HIV-1 RNA and CD4⁺ lymphocyte enumeration than by using each variable alone. Our data suggest that HIV-1 RNA VL peak during the first 6 months after the diagnosis of pediatric HIV infection may be a useful indicator of the risk of disease progression to AIDS or death, independent of the timing of infection and the maternal HIV-1 RNA VL. The mother's HIV-1 RNA level at delivery was also an independent prognostic factor for the pediatric risk of developing AIDS or death, in agreement with previous studies.^{11,40} More surprising, although we were not able to identify significant differences in HIV-1 RNA levels between boys and girls at a given age, progression to AIDS or death seemed to be significantly different by sex and was more rapid for girls than for boys. In adults, lower HIV-1 RNA VLs have been described for women than for men, but the implications of these findings on disease progression remain unclear.⁴¹ Similar sex-specific dynamics of viral replication have been described in children who were followed from birth in the European cohort in which VL peaked higher for girls than for boys until 4 years of age.⁴² In a meta-analysis of late postnatal transmission of HIV-1 in 225 African children, Read et al⁴³ found that the risk

of late postnatal transmission was higher in boys compared with girls. Besides cultural factors, these authors suggested that this association could possibly reflect a greater vulnerability of girls than boys to early HIV-1 infection, eg, infection in utero or around the time of labor and delivery. Our study does not seem to confirm this biological hypothesis because we did not find a significant difference between girls and boys according to the timing of infection.

Our study has several limitations, such as the lack of concomitant CD4⁺ T-cell enumeration in children. Furthermore, the HIV-1 RNA decrease over time may reflect a selection bias, considering the declining number of samples tested during follow-up. Indeed, specimens from healthier children may have been more often available for RNA testing over time. In addition, HIV-1 RNA levels have been obtained retrospectively in frozen, stored plasma. The bDNA assay, however, demonstrated a >4-fold difference in log₁₀ HIV-1 RNA levels in our studied population, suggesting that stored plasma, having undergone 1 freeze-thaw cycle only, was suitable for measurement of HIV-1 VL. All samples but 1 have given concordant results between the quantitative bDNA assay and the qualitative homemade HIV-1 DNA PCR in the context of the predominant CRF02 recombinant AG form in Côte d'Ivoire. Repeated measurements on a given sample could not be performed in our study, missing the assessment of the biological variation in HIV-1 RNA levels for a given child.⁴⁴ From a clinical point of view, 4 children died at stage N without any documented cause. We considered these children rapid progressors, even if their deaths were apparently not HIV related. In addition, the causes of mortality in HIV-1-infected children without reaching CDC category C included mainly respiratory infections and diarrhea. In Côte d'Ivoire as well as in Africa as a whole, these signs/symptoms also occur in HIV-negative children, albeit less frequently.²³ The similarity of the spectrum of the causes of death in HIV-infected children versus those who are not infected was a limit of our study.

CONCLUSIONS

We found that the peaks and slopes of HIV-1 RNA in African HIV-1-infected children are very close to those published from other pediatric cohorts in industrialized countries. Our survey also supports earlier studies performed in the developing world that pediatric HIV-1 RNA VL is predictive of AIDS and death. Given that ART is becoming increasingly accessible in Africa and other developing countries (currently the cheapest cost of ART is approximately 45€ a month in Côte d'Ivoire and free for children through the Ivoirian drug access initiative), the identification of African HIV-1-infected children early in their lives seems crucial to propose to them, if necessary, an ARV therapeutic intervention. Importantly, the quantitative RNA assays have the advantage of providing quantitative plasma HIV-1 RNA levels that can be used to guide initiation and monitoring of ART in children. Finally, there is also a need to care for the mothers as well as their children

because maternal HIV-1 RNA VL has a negative impact on childhood morbidity and mortality.

APPENDIX: ANRS 049 DITRAME STUDY GROUP

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