HIV-1 DNA concentrations and evolution among African HIV-1-infected children under antiretroviral treatment (ANRS 1244/1278)

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Objectives: The objectives of this study were to describe the pretreatment HIV-1 DNA concentrations in children infected with HIV and to evaluate the impact of antiretroviral therapy (ART) on HIV-DNA concentrations.

Methods: This was a retrospective analysis of all children followed up in the ‘Programme Enfant Yopougon’ cohort, Abidjan, Côte d’Ivoire, from 2000 to 2004, who had cryopreserved peripheral blood mononuclear cells (PBMCs) and plasma samples. HIV-DNA was measured using a real-time PCR assay. Mixed-model analysis was used to analyse the factors associated with change in HIV-DNA concentration.

Results: The study included 121 children infected with HIV-1. The median age at inclusion was 6 years (IQR: 3.5–9) and children were at an advanced stage of HIV disease (46.6% and 20.3% presenting CDC stage B and CDC stage C, respectively). At baseline, the median HIV-DNA concentration was 3.4 log10 copies/10^6 PBMCs (IQR: 3.1–3.6). Fifty-four children were initiated on ART during follow-up. After 24 months of ART, HIV-DNA load decreased by 0.32 (IQR: 0.08–0.57) log10 copies/10^6 PBMCs. The only factor associated with the HIV-DNA decrease was a concomitant low HIV-RNA viral load result. Children with efficient ART had a 0.51 log10 copies (IQR: 0.40–0.86) HIV-DNA decrease per million PBMCs.

Conclusions: HIV-DNA concentrations decreased following ART initiation in a large African paediatric cohort. This decline was exclusively associated with the decrease in ongoing replication level achieved. Our study points out that a strong adherence is needed for ART to be efficient on the viral reservoirs, and further reinforces that adherence support is also essential to diminish the reservoir.

Keywords: HIV-DNA, Africa, cohort study

Introduction

The efficacy of early initiation of antiretroviral therapy (ART) in children is well established.1 Several factors limit the scaling-up of paediatric ART, such as the lack of appropriate and palatable combinations, elevated costs and frequent stock shortage of drugs. Lallemant et al.1 called for attention to the specificities of paediatric infection as they preclude direct extrapolation from data gathered in adults. Children have greater CD4 recovery capacity due to more active thymopoiesis, but have a higher rate of incomplete replication suppression.2

In untreated adults, it was shown that HIV-DNA load, a proxy of the viral reservoir, is predictive of disease progression, independently of HIV-RNA and CD4 count.4,5 This predictive value exists at seroconversion regardless of the expression unit [copies/10^6 peripheral blood mononuclear cells (PBMCs), copies/ml].6 HIV-DNA load constitutes the only marker still detectable when replication is undetectable during ART and reflects the past HIV-RNA history.7–10 HIV-DNA decrease has been compared between adults initiating ART at the time of primary or at the time of chronic infection.11

Data on HIV-DNA concentrations in children are scarce, and have mainly been collected from studies in northern countries, often with small sample sizes. HIV-DNA concentration is associated with clinical status, plasma HIV-RNA, CD4 counts, immune activation and HIV-specific immune responses.12–15 In children initiating ART, a higher baseline HIV-DNA is associated with a longer time to achieve HIV-1 RNA undetectability and with virological failure.7–10 The ongoing maturation of the immune system likely induces specific physiopathological mechanisms; studies on HIV reservoirs in children are thus needed. This study aimed at...
improving our knowledge on HIV-DNA in ART-naive children and
dynamics during ART by assessing, for the first time, a biological
parameter that has not been measured in routine care in a
large African paediatric cohort.

**Methods**

All 121 children enrolled in the ‘Programme Enfant Yopougon’ who had
cryopreserved PBMCs and plasma were included in the present study.
The ‘Programme Enfant Yopougon’ was an observational cohort designed
to document morbidity and mortality in HIV-1-infected children in Abidjan,
Côte d’Ivoire (ANRS1244), and to evaluate the impact of ART (ANRS1278).
A total of 282 children were followed from October 2000 to September
2004 at the Yopougon hospital. The procedures and results of the cohort
have been described elsewhere. Briefly, children were eligible if they were
>15 months. Criteria for initiation of ART were CDC stage C or CD4 <15% of
the total lymphocyte count. ART consisted of two nucleoside reverse
transcriptase inhibitors (NRTIs) plus nelfinavir/efavirenz at the physician’s
discretion. Children with CD4 <20% received co-trimoxazole prophylaxis.
The study protocol was approved by the Côte d’Ivoire Ethics Committee on
AIDS and written informed consent was obtained from the children’s par-
ents or caregivers.

Clinical examinations were performed every 3 months. CD4 cell count and
HIV-RNA load were determined every 6 months at the Centre de Diagnostic et
de Recherche sur le SIDA, Abidjan. CD4% was measured by flow cytometry
(FACScan™, Becton-Dickinson). Absolute CD4 counts were determined
to document morbidity and mortality in HIV-1-infected children in Abidjan,
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(FACScan™, Becton-Dickinson). Absolute CD4 counts were determined
using an automated blood cell counter (MaxM Coulter, Beckman). HIV-RNA
load was initially measured prospectively using the Quantiplex HIV-1-RNA
3.0 assay (Chiron) with a detection threshold of 250 copies/mL. From May
2003, HIV-RNA concentrations were determined using the Generic HIV
Charge Virale test (Biocentric) with a detection threshold of 300 copies/mL.
HIV-DNA concentrations were retrospectively quantified in frozen
PBMCs prepared with the same samples by RT–PCR (Generic HIV-DNA
CELL, Biocentric) carried out retrospectively in the virology laboratory of
the Necker Hospital, France. The detection threshold was 70 copies/10^6
PBMCs. Results were reported as HIV-DNA log_{10} copies/10^6 PBMCs or mL, respectively. All but
34 (28.1%) children died during follow-up for whom the last available HIV-DNA was 3.32
(IQR: 2.96 – 3.73) and 3.86 (IQR: 3.48 – 4.9) log_{10} copies/mL, respectively. All but
one child died occurred prior to treatment initiation. The total and
median follow-up durations were 273.5 person-years and
36 months (IQR: 0 – 38).

At baseline, median HIV-DNA was 3.4 log_{10} copies/10^6 PBMCs (IQR: 3.1 – 3.6), or 4.0 log_{10} copies/mL (IQR: 3.6 – 4.3), and did not
differ depending on the CDC stage at inclusion (PBMC–HIV-DNA:

**Results**

One-hundred and twenty-one ART-naive children were included in the
present study, including 65 (53.7%) boys; the median age at
inclusion was 6 years (IQR: 3.5 – 9.0) and 48 children (39.7%) were
≤5 years. Children were mostly at an advanced stage of HIV dis-
ease, with 46.6% and 20.3% of them presenting CDC stage B and
CDC stage C, respectively. Baseline median CD4% was 11.2% (IQR:
5.5 – 17.5). Baseline HIV-RNA load was 5.26 log_{10} copies/mL (IQR:
4.72 – 5.74), and ranged from 5.06 (IQR: 4.50 – 5.52) for CDC stage
A, to 5.29 (IQR: 4.86 – 5.73) for CDC stage B and 5.44 (IQR: 5.05 –
6.23) log_{10} for CDC stage C.

Seven children (5.8%) were lost to follow-up at 24 months.

**Conclusion**

This large African paediatric cohort represents a unique biological
resource for understanding HIV-DNA levels in ART-naive children.

Figure 1. Median log_{10} decrease in HIV-1 DNA concentrations in Ivoirian children receiving ART. (a) All children. (b) Children during efficient ART (censored
at any event among: treatment interruption, missed appointment or HIV-1 RNA >2.4 log copies/mL). Dashed lines represent HIV-DNA per million PBMCs
and dotted lines represent HIV-DNA per millilitre of blood.
CDC stage A, 3.37 (IQR: 3.07–3.61); CDC stage B, 3.41 (IQR: 3.19–3.64); and CDC stage C, 3.29 (IQR: 2.89–3.72). PBMC-HIV-DNA and blood-HIV-DNA concentrations were strongly correlated (Spearman $R = 0.90$, $P < 0.001$).

Before ART initiation, blood-HIV-DNA was independently associated with age [adjusted OR (aOR) =$0.96$, 95% CI =$0.94–0.99$, $P = 0.002$], CD4% (aOR =$1.02$, 95% CI =$1.01–1.03$, $P < 0.001$) and HIV-RNA load (aOR =$1.29$, 95% CI =$1.15–1.46$, $P < 0.001$). PBMC-HIV-DNA was not associated with age, but was associated independently with CD4% (aOR =$1.02$, 95% CI =$1.01–1.03$, $P < 0.001$) and HIV-RNA load (aOR =$1.27$, 95% CI =$1.16–1.40$, $P < 0.001$ and aOR =$1.21$, 95% CI =$1.11–1.32$, $P < 0.001$, respectively).

Fifty-four children (44.6%) initiated ART during follow-up, at a median age of 6 years (IQR: 5–8.5) and were followed for a median duration of 36 months (IQR: 34–42). Forty children (74%) received a nelfinavir-based regimen while 14 received an efavirenz-based regimen. At ART initiation, median CD4% was 9.4% (IQR: 3.7–12.0), median HIV-RNA load was 5.3 log copies/mL (IQR: 4.97–5.73) and median HIV-DNA was 3.4 log10 copies/106 PBMCs (IQR: 3.2–3.7) and 4.0 log10 copies/mL (IQR: 3.7–4.3). After 24 months on ART, median PBMC-HIV-DNA concentration was 3.2 log10 (IQR: 2.8–3.5, $n = 26$) and median blood-HIV-DNA concentration was 3.6 log10 (IQR: 3.4–4.0, $n = 25$).

After 12 and 24 months on ART, PBMC-HIV-DNA decreased by $0.28 \text{log}_{10}$ (IQR: $0.00–0.72$) and $0.32 \text{log}_{10}$ (IQR: $0.08–0.57$), respectively, and blood-HIV-DNA decreased by $0.30 \text{log}_{10}$ (IQR: $0.01–0.73$) and $0.39 \text{log}_{10}$ (IQR: $0.04–0.54$), respectively (Figure 1a).

When limiting analysis to children with efficient ART (i.e. uninterrupted treatment, no missed appointment, HIV-RNA $\leq 2.4 \text{log}_{10}$ at each measurement), PBMC-HIV-DNA decreased by $0.38 \text{log}_{10}$ (IQR: $0.21–0.73$, $n = 20$) and $0.51 \text{log}_{10}$ (IQR: $0.40–0.86$, $n = 8$) after 12 and 24 months, respectively, and blood-HIV-DNA decreased by $0.57 \text{log}_{10}$ (IQR: $0.12–0.73$) and $0.62 \log_{10}$ (IQR: $0.54–0.92$) (Figure 1b).

In multivariate analysis, a low HIV-RNA load during follow-up was the only factor associated with a larger decrease in PBMC-HIV-DNA ($b = 0.11$, 95% CI =$0.08–0.13$, $P < 0.001$) and in blood-HIV-DNA ($b = 0.12$ per log10 HIV-RNA, 95% CI =$0.09–0.15$, $P < 0.001$). Gender, age, protease inhibitor (PI) regimen, CDC stage, CD4%, HIV-RNA load at inclusion or duration on ART were not significantly associated with the decrease in PBMC-HIV-DNA (Table 1) or blood-HIV-DNA.

### Discussion

To our knowledge, this is the first study providing HIV-DNA concentrations in a large African paediatric cohort before and during ART. The baseline PBMC-HIV-DNA concentrations were similar to those described in acute HIV infection in adults [such as the French-PRIMO (3.25 log10)$^4$ and the Ivoirian-PRIMO-CI (3.21 log10)$^5$ cohorts] and were higher than concentrations reported in long-term non-progressors (2.30 log10)$^{18}$ and HIV controllers (1.30 log10).$^{19}$

### Table 1. Mixed model analysis of factors associated with a decrease in HIV-1 DNA load expressed in log copies per 10⁶ PBMCs

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted analysis</th>
<th>Adjusted analysis</th>
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<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>95% CI</td>
</tr>
<tr>
<td>Female gender</td>
<td>0.11</td>
<td>$-0.07, 0.29$</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
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<tr>
<td>$\leq 5$</td>
<td>reference</td>
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<tr>
<td>5–10</td>
<td>$-0.04$</td>
<td>$-0.27, 0.19$</td>
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<tr>
<td>&gt;10</td>
<td>$-0.17$</td>
<td>$-0.43, 0.09$</td>
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<tr>
<td>CDC stage at inclusion</td>
<td></td>
<td></td>
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<tr>
<td>N/A</td>
<td>reference</td>
<td></td>
</tr>
<tr>
<td>B/C</td>
<td>0.10</td>
<td>$-0.10, 0.30$</td>
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<tr>
<td>CD4 cell percentage at inclusion</td>
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<tr>
<td>$&lt;15$</td>
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<tr>
<td>15–25</td>
<td>0.04</td>
<td>$-0.21, 0.28$</td>
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<tr>
<td>ART type</td>
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<tr>
<td>2 NRTIs+non-NRTI</td>
<td>reference</td>
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<tr>
<td>2 NRTIs+PI</td>
<td>0.11</td>
<td>$-0.09, 0.30$</td>
</tr>
<tr>
<td>Current HIV viral load (log$_{10}$ copies/mL)</td>
<td>0.11</td>
<td>$0.08, 0.13$</td>
</tr>
<tr>
<td>HIV viral load at inclusion (log$_{10}$ copies/mL)</td>
<td>$-0.02$</td>
<td>$-0.14, 0.10$</td>
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<tr>
<td>Duration on ART (months)</td>
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<td></td>
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<tr>
<td>6</td>
<td>$-0.24$</td>
<td>$-0.40, -0.08$</td>
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<tr>
<td>12</td>
<td>$-0.30$</td>
<td>$-0.43, -0.16$</td>
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<tr>
<td>18</td>
<td>$-0.47$</td>
<td>$-0.63, -0.31$</td>
</tr>
<tr>
<td>24</td>
<td>$-0.29$</td>
<td>$-0.45, -0.12$</td>
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Before treatment, blood-HIV-DNA was found to decrease with age, but the total stock was increasing with age if the growth of the blood volume was accounted for (results not shown). In contrast, PBMC-HIV-DNA was associated with CD4% and HIV-RNA but was not related to age. These results highlight child specificities such as a higher thymic output of naive CD4 cells in contrast to expansion of memory T cells in adults, and age-dependent hyperlymphocytosis in contrast to fixed levels in adults.20 Unfortunately our sample size did not allow us to assess the predictive value of HIV-DNA before treatment initiation for clinical and mortality outcomes.

This study showed that HIV-DNA concentrations decreased during efficient ART, i.e. when an undetectable HIV-RNA load was achieved. This finding is consistent with those published in adults and children. For instance, our results are similar to those of Saitoh et al.,12 which showed a rapid decline in HIV-DNA followed by a plateau reached at 18 months after ART initiation.

In our study, HIV-RNA load obtained during ART was the only factor correlated with the HIV-DNA decrease, whereas De Rossi et al.8 found that a higher baseline HIV-RNA correlated with a higher HIV-DNA early decay.

Our study has limitations. Children were almost exclusively ≥2 years. The rapid progressors were likely not represented. The potency of the most commonly used regimen in our study (two NRTIs plus unboosted nevirapine) might be expected to be less than that of efavirenz or other contemporary regimens, including boosted PI-based regimens. These might thus yield different results.

In conclusion, our study revealed evidence that ART lowered HIV-DNA concentrations in African treated children but underlines the difficulty of diminishing viral reservoir in real life in African children. In our era, a large number of HIV-infected children will reach adulthood, requiring strategies to diminish their viral reservoir and improve their outcomes. Our study underscores that a strong adherence is necessary to achieve an undetectable viral load and further reinforces that adherence support is also essential to diminish the viral reservoir.

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Transparency declarations
None to declare.

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