Escalating and sustained immunovirological dissociation among antiretroviral drug-experienced perinatally human immunodeficiency virus-1-infected children and adolescents living in the Central African Republic

A STROBE-compliant study

Christian Diamant Mossoro-Kpinde, MD, PhD, MSc, Jean-Chrysostome Gody, MD, Jean De Dieu Longo, MD, PhD, Gérard Grésenguet, MD, PhD, Joël Fleury Djoba Siawaya, MD, PhD, Laurent Bélec, MD, PhD, MPH, MSc

Abstract

Sub-Saharan Africa has the vast majority (~90%) of new pediatric acquired immunodeficiency syndrome cases worldwide. Biologically monitoring HIV-infected pediatric populations remains challenging. The differential interest of human immunodeficiency virus (HIV)-1 RNA loads and CD4 T-cell counts is debated for the treatment of pediatric acquired immunodeficiency syndrome patients.

Long-term antiretroviral treatment (ART) outcomes regarding immunological and virological surrogate markers were longitudinally evaluated between 2009 and 2014 (over 57 months) in 245 perinatally HIV-1-infected children and adolescents born from HIV-infected mothers, treated at inclusion for at least 6 months by the World Health Organization-recommended ART in Bangui, Central African Republic.

Patients were monitored over time biologically for CD4 T-cell counts, HIV-1 RNA loads, and drug resistance mutation genotyping. Children lost to follow-up totaled 6%. Four categories of immunovirological responses to ART were observed. At baseline, therapeutic success with sustained immunological and virological responses was observed in 80 (32.6%) children; immunological and virologic nonresponses occurred in 32 (13.0%) children; finally, the majority (133; 54.2%) of the remaining children showed discordant immunovirological responses. Among them, 33 (13.4%) children showed rapid virological responses to ART with an undetectable viral load, whereas immunological responses remained absent after 6 months of treatment and increased progressively.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

The authors have no conflicts of interest to disclose.

© 2020 the Author(s). Published by Wolters Kluwer Health, Inc.
1. Introduction

Perinatal human immunodeficiency virus type (HIV-1) infection remains one of the leading causes of child mortality in sub-Saharan Africa.\[1\]-[2] Indeed, infants infected perinatally have poor survival prognoses, without antiretroviral treatment (ART), more than half of them do not reach their second birthday.\[3\] Since 2000, child mortality associated with HIV in sub-Saharan Africa has been significantly reversed.\[1\] Globally, 1.8 million (1.3 million–2.4 million) children (aged <15 years) had HIV in 2017, mainly in sub-Saharan Africa (88.3%), and 328 deaths occurred daily.\[1,4,5\]

In addition to the significant increase in the life expectancy of HIV-1-infected children receiving ART in sub-Saharan Africa,\[6\] the prolonged use of antiretroviral drugs has raised the question of its long-term impact on children’s quality of life.\[6-10\] Likewise, several studies have reported on the outcomes of antiretroviral therapy in African pediatric populations,\[11\] but few of them have concerned virological and immunological long-term outcomes.\[12-23\] Interestingly, the correlation between immunological and virological failures in pediatric cohorts has often appeared minimal,\[16-17,21\] suggesting that the surrogate markers — HIV-1 RNA loads and CD4 T-cell counts — provide complementary rather than exclusive information for adapting ART for children.

To evaluate the management of pediatric acquired immunodeficiency syndrome (AIDS), an observational cohort of HIV-infected children has been followed up since 2007 at the Complexe Pédiatrique in Bangui, Central African Republic, the main health care clinic for HIV-infected children in the country.\[26-28\] where an HIV epidemic was once considered “out of control.”\[29,30\] The challenge of biologically monitoring HIV-infected pediatric populations in sub-Saharan Africa, with concerns about the differential interest of HIV-1 RNA loads and CD4 T-cell counts in caring for pediatric AIDS patients, prompted us to assess for the first time long-term prospective immunological and virological outcomes in a cohort of HIV-1-infected children attending the Complexe Pédiatrique and receiving an ART regimen adapted according to successive World Health Organization (WHO) guidelines.\[31-33\]

2. Material and methods

2.1. Study population

HIV-1-infected children followed up at the Complexe Pédiatrique in Bangui were prospectively recruited from May 2009 and followed up for 57 months until 2014 in a descriptive observational cohort study assessing their immunological and virological outcomes following ART. All the included children were born from HIV-1-infected mothers who were under ART for the prevention of mother-to-child transmission according to the national guidelines. Newborn children infected by HIV-1 despite prevention strategies were followed up and cared for according to the WHO recommendations for resource-limited countries.\[31,32\]

The inclusion criteria were as follows:

1. Having received ART for at least 6 months, consisting of first- or second-line regimens as recommended by WHO guidelines;\[31-34\]
2. Availability of simple demographic data on children (eg, age and gender) and treatment history (eg, duration of treatment and therapeutic line); and
3. Informed consent from each child’s biological parent(s) or guardian(s).

The following definitions for children and adolescents were used according to the 2015 revised WHO recommendations.\[36\] A child is an individual between 1 and 10 years old, and an adolescent (ie, “teenager”) is between 10 and 19 years old.

2.2. Plasma HIV-1 RNA loads and CD4 T-cell counts

Venipuncture Ethylene-diamine tetra-acetic acid blood samples were obtained from each included child both at inclusion and every 12 months during the follow-up period, according to the 2013 WHO recommendations.\[34\]

Plasma HIV-1 RNA load and CD4 T-cell measurements were carried out as previously described.\[38\] In brief, plasma HIV-1 RNA loads were measured at the Laboratoire National de Biologie Clinique et de Santé Publique in Bangui, using the Amplicx platform developed by Biosynex (Strasbourg, France), which integrates a fully automated station for nucleic acid extraction (RNA and/or DNA) with a real-time polymerase chain reaction amplification station, using lyophilized Amplicx HIV-1 RNA quantitative reagents (Biosynex). The assay detects HIV-1 groups M and O and several circulating recombinant forms.\[37\] The Laboratoire National de Biologie Clinique et de Santé Publique participates in an external quality assurance testing program organized by the virology laboratory of the Hôpital Européen Georges Pompidou in Paris, France. The CD4 T lymphocyte count was carried out using the Apogee auto 40 flow...

According to the 2013 WHO recommendations, the threshold for virological failure (VF+) was set at 1000 copies/mL.\[34\] This threshold was further consolidated by WHO in 2014\[35\] and 2016,\[36\] and that it continues to be used.\[37\]

Interlaboratory external quality control of the molecular and flow cytometry platforms was performed regularly using samples provided by the Hôpital Européen Georges Pompidou in Paris.\[28,37,40,41\]

2.3. Detection of drug resistance mutations (DRMs)

Detection of DRMs was carried out both at inclusion and after 39 months of follow-up (in 2013), as previously described.\[28\] In brief, aliquots of plasma were obtained at inclusion and follow-up, kept frozen at –80°C until being sent in a dry ice box to the virology unit of the Hôpital Européen Georges Pompidou in Paris, and then kept frozen at –80°C until their processing for resistance mutation genotyping. Antiretroviral resistance genotyping was performed on plasma specimens from children with a detectable HIV-1 RNA load. HIV-1 protease and reverse transcriptase pol genes were sequenced using the ViroSeq HIV-1 genotyping system (Celeria Diagnostics, Alameda, CA). Resistance mutations were reported and interpreted based on the Agence Nationale de Recherches sur le SIDA et les Hépatites Virales algorithm (updated in September 2016; http://www.hivfrenchresistance.org). Protease and reverse transcriptase sequences were submitted to the European Nucleotide Archive with the following accession numbers: LT577626 to LT577763 and LT726745 to LT726792 (http://www.ebi.ac.uk/ ena/data/view/). At baseline, antiretroviral resistance genotyping was carried out on the plasma samples of 125 patients with a detectable plasma HIV-1 RNA viral load. At follow-up, antiretroviral resistance genotyping was carried out on 58 plasma samples randomly selected from those of nearly half of 133 children with a detectable plasma HIV-1 RNA viral load; 50 plasma samples were obtained from children receiving first-line regimens, and 8 were from children receiving second-line regimens.

2.4. Classification according to immunovirological outcomes

A successful immunological response to therapy (I+) was defined as an increase in the CD4 T-cell count according to age at the follow-up visit (ie, CD4 T-cell count >750/µL for children younger than 5 years and CD4 T-cell count >500/µL for children and adolescents older than 5 years).\[33,34\] A virological response to treatment was defined as achieving a plasma HIV-1 RNA load below the limit of detection (ie, less than 20 copies/mL or 1.3 log copies/mL). Thus, virological responders (V+) were the children with an undetectable HIV-1 RNA load.

Finally, the study participants were classified into 4 categories according to their immunovirological responses: [I+, V+] for both immunological and virological responders, [I+, V−] for immunological nonresponders but virological responders, [I+, V−] for immunological responders but virological nonresponders, and [I−, V−] for both immunological and virological nonresponders.

2.5. Ethics statement

This study was formally approved by the Scientific Committee of the Faculté des Sciences de la Santé in Bangui, constituting the National Ethical Committee (Reference #2UB/FACSS/CSVPR/09) in the Central African Republic. Informed written consent was obtained from mothers for themselves and on behalf of their respective child participating in the study.

2.6. Statistical analyses

Paired patient data collected at inclusion and after the follow-up period were compared using the $\chi^2$ test for categorical variables and unmatched Student $t$ test for quantitative variables. Means and proportions were estimated with their 95% confidence intervals.

3. Results

Full descriptions of the pediatric cohort have been reported in 2009\[27\] and 2013.\[28\] The therapeutic regimen changed only slightly between 2009 and 2014, according to particular changes in successive WHO recommendations\[31–35\], the progressive suppression of stavudine (d4T) and the introduction of tenofovir disoproxil fumarate.

3.1. Baseline characteristics of study children

At inclusion, 245 HIV-1-infected children were prospectively recruited within a period of 3 months (Table 1). The median age of the children was 9.1 years (range: 1–16 years), and the sex ratio (male/female) was 0.87 (114/131). All the children were already under ART with the majority ($n=230; 93.9\%$) under a first-line ART regimen according to WHO recommendations\[31,32\] for a mean duration of 18.6 months (range: 7.9–29.2 months). The remaining 15 (6.1\%) children were under a second-line ART regimen for a mean duration of 31.2 months (range: 9.8–52.7 months). As recommended in the national guidelines and by the WHO in 2009\[31,32\] at inclusion, the most prescribed treatment was the combination of d4T/lamivudine/nevirapine. In 2009, only a minority [$18/245 (7.3\%$)] of the children were under a protease inhibitor (PI)-based regimen.

3.2. Baseline immunological and virological outcomes

Figure 1 depicts the classification of the 245 study children into 4 groups: [I+, V+], [I−, V−], [I+, V−], and [I−, V−], according to their immunovirological responses to treatment at baseline. Plasma HIV-1 viral loads were detectable (V+) in 132 study children (53.8\%) but undetectable (V−) in 113 (46.2\%) (Table 1). These 2 groups of children (V− and V+) were each divided into 2 subgroups according to their immunological response as follows: The first subgroup [I+, V−] comprised 80 (32.6\%) children who were immunological and virological responders with an undetectable viral load and a mean CD4 T-cell count estimated at 1063 cells/µL. The 32 children (13.0\%) in the second subgroup [I−, V−] were immunological and virological nonresponders, with a very low mean CD4 T-cell count (mean: 226 cells/µL and high viral load (mean: 4.7 log copies/mL). More than half of the study cohort (133 [54.2\%]) children showed immunological and virological discordant responses to treatment. Among them, 100 (40.9\%) were immunological responders but virological nonresponders [I+, V−] with a high CD4 T-cell count (mean: 809 cells/µL) and high viral load (mean: 4.8 log copies/mL). The remaining 33 (13.5\%) discordant children were immunological nonresponders but virological responders [I−, V−].
with a low CD4 T-cell count (mean: 314 cells/μL) and undetectable viral load.

Among the 125 successful genotypes, 94 were isolated from the children who were immunological responders but virological nonresponders, 91% of the children harbored viruses with at least 1 resistance mutation to antiretroviral drugs (Fig. 2). All (100%) the children in the subgroup of immunological nonresponders but virological responders harbored viruses with at least 1 resistance mutation to antiretroviral drugs.

In the subgroup of immunological nonresponders [I+, V-], 94% of the children harbored viruses with at least 1 resistance mutation to antiretroviral drugs (Fig. 2 and Table 1).

Overall, at baseline, about 10% (12/125) of the patients with a detectable viral load harbored viruses genotypically resistant to the 3 molecules included in the main prescribed antiretroviral combination (d4T/3TC/EFV) (Fig. 3).

### 3.3. Longitudinal outcomes of study children after temporal follow-up

In 2014, d4T was progressively replaced with tenofovir disoproxil fumarate, and 12.7% (28/220) of children were under a PI-based regimen.

As depicted in Figure 1, 10% (25/245) of the study patients were lost to follow-up, whereas most (220/245, 90%) of the included children at baseline were followed up prospectively until 2014. Among them, 54% were girls (119/220) with a median age of 13.8 years (range: 5–18 years). The majority of the children and adolescents (n=198; 90%) received a first-line regimen according to the revised 2013 WHO recommendations, for a mean duration of 65.6 months (range: 18.8–69.9 months) for first-line treatment. The remaining children and adolescents (n = 22; 10%) received a second-line regimen for a mean duration of 78.2 months (range: 13.3–88.3 months).

### Table 1

**Clinicobiological characteristics of HIV-1-infected children followed up at the Complexè© Pédiatricå© in Bangui, at inclusion (n = 245) and after 57 mo of immunovirological monitoring and therapeutic follow-up (n = 220).**

<table>
<thead>
<tr>
<th></th>
<th>Inclusion (n = 245)</th>
<th>Follow up (n = 220)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[I+, V+]</td>
<td>[I+, V+]</td>
</tr>
<tr>
<td></td>
<td>[I+, V+]</td>
<td>[I+, V+]</td>
</tr>
<tr>
<td></td>
<td>[I+, V+]</td>
<td>[I+, V+]</td>
</tr>
<tr>
<td></td>
<td>[I+, V+]</td>
<td>[I+, V+]</td>
</tr>
<tr>
<td>Number of patients who were immunological responders or virological responders or nonresponders at baseline</td>
<td>80 (32.6)±2</td>
<td>66 (30)±2</td>
</tr>
<tr>
<td>Number of children who were immunological responders or nonresponders or virological responders or nonresponders at follow-up</td>
<td>80 (32.6)±2</td>
<td>66 (30)±2</td>
</tr>
<tr>
<td>Number of children in first- or second-line regimens included in each of the 4 immunovirological subgroups at baseline and follow-up</td>
<td>80 (32.6)±2</td>
<td>66 (30)±2</td>
</tr>
<tr>
<td>Number of drug resistance genotypes harboring resistance to one or more WHO-recommended drugs</td>
<td>80 (32.6)±2</td>
<td>66 (30)±2</td>
</tr>
<tr>
<td>Adherence [%], mean ±σ, (range)</td>
<td>91.7±2.6 (89.4–93.8)</td>
<td>91.2±3.4 (88.9–93.6)</td>
</tr>
<tr>
<td>Treatment duration [yr, mean ±σ, (range)]</td>
<td>1.3±0.6 (1.1–1.5)</td>
<td>1.3±0.6 (1.1–1.5)</td>
</tr>
<tr>
<td>Viral load [log copies/mL, mean ±σ, (range)]</td>
<td>31.8±3.4 (29.9–33.8)</td>
<td>31.8±3.4 (29.9–33.8)</td>
</tr>
<tr>
<td>CD4 T cell count [cells/μL, mean ±σ, (range)]</td>
<td>508±123 (428–613)</td>
<td>508±123 (428–613)</td>
</tr>
<tr>
<td>Lost-of-follow-up [n/Vn, (range)]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Total number of resistance to WHO-recommended drugs [n, (%)]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DRMs to PI [n, (%)]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DRMs to NNRTI [n, (%)]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DRMs to NRTI [n, (%)]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DRMs to NNRTI and NRTI [n, (%)]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DRMs to NRTI or NNRTI and PI [n, (%)]</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Number of patients who were immunological responders or virological responders or nonresponders at baseline.
*Number of children who were immunological responders or nonresponders or virological responders or nonresponders at follow-up.
*Number of drug resistance genotypes harboring resistance to one or more WHO-recommended drugs.
Although none of the 4 subgroups of patients were spared, most of the children lost to follow-up (15/25; 60%) were in the group of immunological and virological nonresponders [I/C0, V/C0] (Fig. 1 and Table 1).

Plasma HIV-1 viral load measurements yielded high rates (60%) of virological nonresponders (V/C0), most of whom (96%) were in virological failure according to the WHO criteria (ie, circulating viral load above 1000 copies/mL) (Fig. 2). Long-term immunological responses to ART enabled refining the biological responses to ART. Thus, the longitudinal landscape of the children cohort remained over time almost the same as that at baseline, with 57.8% (127/220) of the patients showing discrepant responses to treatment ([I+, V-] → [I+, V+]), 30.0% (66/220) in therapeutic success ([I+, V+]), and 12.2% (27/220) with unfavorable immunovirological profiles ([I-, V-]) (Fig. 1 and Table 1).

Most of the immunological and virological responder children (55/76; 72.3%) at baseline consistently remained in full therapeutic success after 5 years of ART; only 3 (3.9%) of them experienced therapeutic failure after the follow-up period; and the remaining immunological and virological responder children at baseline (18/76; 23.7%) showed immunological and virological discrepant responses to treatment at follow-up: [I+, V+] → [I-, V-]; 6 (7.9%) and [I+, V+] → [I+, V+]: 12 (15.8%).

The majority (16/17; 94.1%) of the immunological and virological nonresponder children in therapeutic failure at baseline still failed to restore their CD4 count and viral load ([I-, V-] → [I+, V-]). Only 1 (5.8%) of them succeeded in controlling viral replication ([I+, V-] → [I+, V+]), and the remaining 2 (2.0%) children in this subgroup experienced therapeutic failure with unfavorable immunovirological responses ([I+, V+] → [I+, V+]).

Regarding the discordant children [I-, V+] at baseline, 46.7% (14/30) of them remained in the same subgroup, 33.3% (10/30) evolved to immunological and virological responses with therapeutic success [I+, V+], and 20.0% (6/30) experienced therapeutic failure with unfavorable immunovirological responses ([I-, V+] → [I-, V-]).

After 39 months of follow-up, 96% (56/58) of the 58 genotypes from the child cohort with detectable viral loads (V/C0) and/or in virological failure according to the 2013 revised WHO criteria (V/C0 or VF+) harbored at least 1 DRM (DRM+) (Figs. 2 and 3). The distributions of DRMs in the virological nonresponders and in the patients in virological failure were similar, with a minority showing DRMs to PIs and around half of them exhibiting DRMs to NRTIs or NNRTIs. As expected, a high frequency of natural polymorphisms in the protease gene sequences was observed in over 90% of the sequenced viruses.

Regarding the NRTI class, nearly half of the nonresponder patients (V/C0) displayed viruses that harbored at least 1 DRM associated with NRTI resistance (Table 1). At follow-up, most of the sequenced viruses remained susceptible to the majority of the NRTIs, NNRTIs, and PIs proposed for the WHO-recommended second-line regimen [33,34] (not shown).

### 3.4. Distribution of children showing discordant immunovirological responses [I+, V+] over time

In 2014, children showing discrepant immunological and virological responses to treatment represented 58% (127/220) of the cohort (Fig. 1 and Table 1).
A significant proportion of them (106/127; 83.5%) were immunological responders but virological nonresponders \([I^+, V^-]\) with a high CD4 T-cell count (mean: 782 cells/\mu L; range: 552–1011 cells) and viral load (mean: 4.6 log copies/mL; range: 2.3–5.9 log copies).

The longitudinal follow-up of children showing virological failure but immunological responses \([I^+, V^-]\) revealed a significant increase in this subgroup of children, from 40.9% (100/245) at baseline to 48.2% (106/220) after 57 months of follow-up with an annual growth rate of 1.23%.

At baseline, 91% (91/100) of the discordant \([I^+, V^-]\) children were treated with a first-line regimen; after a mean duration of 76.7 months (range: 9.7–80.8 months) of therapeutic follow-up, most of them continued their first-line treatment, and only 3 (2.8%) of them were switched to second-line therapy (Table 1).

The majority (91%) of the sequenced viruses from discordant \([I^+, V^-]\) children at baseline were resistant to WHO-recommended antiretroviral drugs (Fig. 2 and Table 1). All (100%) the sequenced viruses from discordant \([I^+, V^-]\) children at follow-up harbored genotypic resistance. Although there was a slight decrease in the mean viral load of these patients during follow-up (4.8 log copies/mL at baseline to 4.6 after follow-up), this variation was not statistically significant \((P = .13)\) (Fig. 4 and Table 1). Likewise, there was no statistically significant change in CD4 T-cell counts over time in the \([I^+, V^-]\) discordant children \((P = .6)\) (Fig. 4). Furthermore, 55 children (mean age: 11 years; range, 4–18 years) among the 94 children (mean age: 12 years; range, 4–18 years) remaining in the group \([I^+, V^-]\) during the follow-up showed a significant decrease in their mean CD4 T-cells count overtime (916.1 cells/\mu L at baseline vs 784.7 cells/\mu L after follow-up \((P < .01)\)) (Fig. 1), while their CD4 T-cells counts remained all above the thresholds of immunological failure according to age (ie, CD4 T-cell count >750/\mu L for children less than 5 years and >500/\mu L for children and adolescents older than 5 years).

There was also a slight decrease in the mean viral load of the \([I^-, V^-]\) patients during follow-up, without statistical significance \((P = .31)\) (Fig. 4).

4. Discussion
In this study, the long-term WHO-recommended ART was longitudinally evaluated using immunological and virological surrogate markers in 245 perinatally HIV-1-infected children and
adolescents born from HIV-infected mothers and followed up for 57 months at the Complexe Pédiatrique in Bangui. Children lost to follow-up totaled only 6%, indicating a high retention rate. Strong heterogeneity was observed in the immunological and virological responses to ART with the identification of 4 categories of immunovirological responses: full therapeutic success with sustained immunological and virological responses to treatment in nearly one-third of children, full therapeutic failure with immunological and virological nonresponses in nearly one-tenth of children always associated with high levels of DRMs, and discordant immunovirological responses in the majority (≈60%) of the remaining children.

Immunological and virological responses [I+, V+] with restoration or maintenance of normal CD4 T-cell counts and suppression of plasma HIV-1 RNA loads were observed in one-third of the cohort children. Our findings are consistent with those of previous reports on the frequent effectiveness of ART in HIV-infected children living in resource-limited countries, mainly in sub-Saharan Africa.[20,43–49] These observations justify the necessity for biologically monitoring affected children and adolescents at least once a year, especially for assessing their HIV-1 RNA load, regardless of their CD4 T-cell count, to enhance treatment at the slightest suspicion of treatment failure.[13,34,39]

The group of children in full therapeutic failure [I−, V−], in whom the plasma HIV-1 RNA load was elevated over time and the CD4 T-cell count remained persistently low after the follow-up period, represented slightly more than one-tenth of the cohort. We defined therapeutic failure according to the nonresponses of the 2 immunological and virological surrogate markers, which may explain the apparently low rate of treatment failure below the range of 19.7% to 53.0% in sub-Saharan Africa.[10,12,25,44–56] Furthermore, this group was strongly associated with a high rate of patients lost to follow-up (47%), which could indicate the final rate of therapeutic failure. The frequent absence of being switched to a second-line regimen, despite a persistent detectable viral load in the long-term first-line-treated study children, would have likely led to an increased rate of virological failure, thus reinforcing the group of full therapeutic failure.[10,11,15,56,57] Only 1 patient in the [I−, V−] group was moved to the discordant immunological nonresponders group [I+, V−] after the follow-up. Indeed, it is well documented that HIV-1 induces rapid depletion of CD4 T cells before ART initiation.[58–60] Providing low CD4 T-cell count recovery in patients starting ART with severe immune depression.[61]

More than half (58%) of our cohort exhibited unexpected discrepant responses to ART after nearly 5 years of follow-up, indicating that the correlation between immunological and virological failures was minimal, as previously observed in African pediatric cohorts.[21,24] Discordant immunological and

Figure 3. Percentage of genotypes harboring mutations conferring resistance to the major WHO-recommended antiretroviral drugs at baseline (n = 245) and after 39 mo of ART (n = 220) according to the therapeutic lines in HIV-1-infected children born from HIV-positive mothers and cared for at the Complexe Pédiatrique in Bangui. Genetic analysis of the 58 HIV-1 pol sequences obtained in a 2013 follow-up showed broad genetic diversity. Thus, most children were infected with CRF11_cpx (34.4%) and CRF01-AE (18.9%), or with HIV-1 subtype A (12.1%). Furthermore, a wide variety of HIV-1 subtypes were observed: 5.1% each for CRF02_AG, CRF15_cpx, H, K, and 3.4% each for CRF15 and subtype P1 and B. Finally, with the lowest proportion (1.7%) subtypes C and G. ART = antiretroviral treatment, CRF = circulating recombinant form, HIV = human immunodeficiency virus, WHO = World Health Organization.
Figure 4. Variation over 57 mo of monitoring mean CD4 T-cell counts (A) and plasma HIV-1 RNA loads (B) according to the subgroups of immuno-virological responses to ART in HIV-1-infected children treated according to WHO recommendations. Discordant [I+, V+] children showed a decrease (Δ = 0.2) in the mean HIV-1 RNA load, although the variation over time was not statistically significant (P = 0.13). ART = antiretroviral treatment; HIV = human immunodeficiency virus; WHO = World Health Organization.

Virological responses under ART have been reported for adults and children in Western countries and resource-limited settings. At inclusion, a small number of study children (13.0%) belonged to the group of immunological nonresponders but virological responders [I+ , V−]. Although these patients failed to control HIV-1 viral replication after the follow-up period and thus were all in virological failure with viruses harboring high rates of DRMs (100%), their CD4 T-cell counts remained persistently high, above normal levels. An increased CD4 T-cell count, higher than the WHO threshold for immunological success without suppressing viral loads that demonstrate discordant responses, has been reported. The following hypotheses can be considered. First, the recovery of thymic function and high thymic output under ART favor the immunorestoration of HIV-1-infected children with a sustained CD4 T-cell increase, despite the persistence of viral replication. Second, partial viral suppression under certain antiretroviral drug regimens (mainly PI) may reduce CD4 T-cell turnover and activation, thereby resulting in sustained CD4 T-cell gains, despite detectable viral replication. However, only a minority of children in the [I+, V−] group in the study cohort received long-term ART containing PI. Third, about 5% of the children in the discordant [I+, V−] group harbored viruses exhibiting a resistance mutation (V82A in protease) previously reported in discordant patients. Fourth, discordant responses during ART may be related to HIV-directed immune responses, diminished cellular activation, and preservation of non-syncytium-inducing viruses, as previously reported in adults. Fifth, the accumulation of high levels of DRMs over time could have provided impaired viral fitness with lower viral replication capacities. Furthermore, the longitudinal observation of our cohort children who were in virological failure but immunological responders [I+, V−] revealed a progressive and statistically significant increase over time, with a growth rate of 1.53% per year, suggesting selective advantages for the virus as well as for the discordant children, who appeared unexpectedly tolerant to the virus. Finally, our observations suggest that the discordant [I+, V−] group poses crucial concerns about therapeutic options, as previously discussed. Maintaining such children in the current line certainly enables placing therapeutic pressure on potentially defective viruses or viruses with diminished fitness, which would lead despite resistance to a positive therapeutic effect. Integrase strand transfer inhibitors...
may constitute relevant therapeutic alternatives as second- and third-line ART regimens for HIV-1-infected children and adolescents in therapeutic or virological failure living in sub-Saharan Africa.\[93\] In conclusion, basing treatment decisions exclusively on immunological parameters would lead to unnecessary treatment switches in a substantial number of patients, as previously emphasized.\[92,94\] Thus, close biological monitoring with access to routine plasma HIV-1 RNA load and CD4 T-cell count monitoring is crucial, despite being difficult in resource-constrained countries, and constitutes a strong necessity for adapting the complex outcomes of ART in HIV-1-infected children born from infected mothers.

Acknowledgments

We are particularly grateful to Dr Alexia Naissem and Mr Dionke Fofana from the Ensemble pour une Solidarité Thérapeutique en Réseau (Paris, France) and Expertise France, Paris, for their contributions and relevant discussions. We thank Miss Rosine Feissona for her excellent technical assistance. We thank Dr Thomas Lamy of Biosynex, for providing the kits for the HIV-1 RNA load measurements used in this study. We thank Dr Pierre Roques, Commissariat à l’Énergie Atomique, Division of Immuno-Virologie, Institute of Emerging Diseases and Innovative Therapies, Fontenay-aux-Roses, France, for HIV-1 pol sequence analyses and GenBank submission.

Author contributions

Conceptualization: Christian Diamant Mossoro-Kpinde, Jean Chrysostome Gody, Ralph-Syndey Mboumba Bouassa, Laurent Bélec.

Data curation: Sandrine Moussa, Jean De Dieu Longo.

Formal analysis: Christian Diamant Mossoro-Kpinde, Ralph-Syndey Mboumba Bouassa, Sandrine Moussa, Mathieu Matta.

Investigation: Laurent Bélec.

Methodology: Ralph-Syndey Mboumba Bouassa.

Project administration: Jean Chrysostome Gody.

Supervision: Jean De Dieu Longo, Gérard Grésenguet, Joël Fleury Djioja Siawaya, Laurent Bélec.

Validation: Jean De Dieu Longo, Gérard Grésenguet, Joël Fleury Djioja Siawaya, Laurent Bélec.

Writing – original draft: Christian Diamant Mossoro-Kpinde, Ralph-Syndey Mboumba Bouassa, Mohammad-Ali Jenabian, Hélène Péré, Charlotte Charpentier, Laurent Bélec.

Writing – review & editing: Christian Diamant Mossoro-Kpinde, Ralph-Syndey Mboumba Bouassa, Hélène Péré, Charlotte Charpentier, Joël Fleury Djioja Siawaya, Laurent Bélec.

References


