Outcomes of protease inhibitor-based antiretroviral therapy amongst children and associated-factors in Yaoundé, Cameroon

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Abstract

Background

There are limited data on protease inhibitor (PI)-based antiretroviral therapy (ART) amongst children in resource-limited settings, for informing on optimal paediatric regimens.

Objective

To evaluate therapeutic response to PI-based ART amongst HIV-infected Cameroonian children.

Methods

A retrospective study was conducted amongst children aged 2–18 years receiving a PI-based ART at the Essos Hospital Centre (EHC), Yaounde, Cameroon. Primary end points were therapeutic success on PI-based ART, defined as clinical success (WHO I/II clinical stage), immunological success (CD4 ≥ 500/mm³) and viral suppression (viral load [VL] <1000 copies/ml). Factors associated with therapeutic success were assessed in uni- and multivariate analysis using SPSS software v.2.0; with p<0.05 considered statistically significant.

Results

A total of 71 eligible children on PI-based ART were enrolled (42 on initial and 29 on substituted regimens), with a median age of 8 [IQR: 5–12] years and mean duration on ART of 7 years. Following therapeutic responses, all (100%) experienced clinical success, 95.2% experienced immunological success (91.7% on initial and 97.2% on substituted PI/r-based regimens) and 74.7% viral suppression. In univariate analysis, viral suppression was
associated with: younger age (p<0.0001), living with parents as opposed to guardians (p = 0.049), and the educational level (p<0.0001). In multivariate analysis, only the age ranges of 10–14 years (OR: 0.22 [0.07–0.73]) and 15–18 years (OR: 0.08 [0.02–0.57]), were determinants of poor viral suppression.

**Conclusion**

Among these Cameroonian children, PI-based ART confers favourable clinical and immunological outcomes. The poor rate of viral suppression was mainly attributed to adolescence (10–18 years).

**Introduction**

Almost 1.8 million children are living with HIV (CLHIV) worldwide, of whom 1.6 million are from sub-Saharan Africa (SSA) [1,2]. During the last decade, the increasing access of antiretroviral therapy (ART) has improved the survival rate amongst CLHIV in SSA, with about 50% paediatric ART coverage [2,3]. Progress in the therapeutic management of CLHIV in SSA has ensured the revision of eligibility criteria both for initiating first-line and for switching to second-line ART regimens following the world health organisation (WHO) recommended public health approach [4,5]. Of note, based on recent evidence and the effective implementation of prevention of mother-to-child transmission (PMTCT) option B+ in SSA settings, current guidelines recommend ART regimens consisting of ritonavir-boosted protease-inhibitor (PI/r) as the preferred first line option in children below 3 years, and as preferred second-line option after failure to a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART regimen [5].

In spite of the effectiveness of current ART strategy in both adult and children populations, achieving the expected target for viral suppression (i.e. 90% viral load below 1,000 copies/mL) among children is more challenging compared to adults [6]. This is particularly true in the frame of very high viral loads in paediatric populations, limited paediatric therapeutic options, the wide use of drugs with low-genetic barriers to resistance (i.e. the majority of CHIV still receiving NNRTI-based regimens) and the paucity of evidence on response to PI-based regimens either as first- or second-line ART in SSA [6,7]. As current efforts in viral monitoring of CLHIV would increase the switch to PI/r-based regimens, it becomes crucial to set-up relevant strategies for: (a) ensuring a long-term successful initial regimen, (b) ensuring viral re-suppression once on second-line regimen; and (c) understanding the local factors associated with treatment outcomes [7].

Amongst Cameroonian CLHIV, findings revealed poor therapeutic response, especially during adolescence, and high rates of acquired HIV drug resistance (HIVDR) among those failing NNRTI-based ART [8,9]. Of note, this high rate of resistance was favoured by a prolonged exposure to failing regimens, which in turn prompts the accumulation of DR mutations [10,11]. Thus, in the frame of limited knowledge about response to paediatric PI/r-containing regimens, our study objectives were to evaluate the therapeutic (clinical, immunological and virological) response of children receiving a PI/r-based ART, to compare the response on PI/r used in first- versus second-line combinations, and study the determinants of therapeutic response.
Materials and methods

Study design and site description

A retrospective cohort-study was conducted amongst children aged 2–18 years receiving PI/r-based regimens either as initial (i.e. first-line) or substituted (i.e. second-line) ART at the pediatric department of the Essos Hospital Centre (EHC) in Yaoundé, the capital city of Cameroon, from 2005 to 2016.

The EHC is an approved treatment centre for HIV-infection in adults, adolescents and children; and paediatric ART was launched onsite by 2005. At this study site, CLHIV on PI/r-based regimens receive at early age a syrup of ritonavir boosted with lopinavir (LPV/r), while those at older age or weighting >10kg received pills of LPV/r. Detailed site description is provided elsewhere [8,9].

Study participants and sampling procedure

Following an exhaustive sampling at the study site (EHC), all CLHIV fulfilling the following criteria were enrolled in the study: (a) aged 2–18 years, (b) currently receiving a PI/r-based ART regimen, (c) treated for at least 6 months with a PI/r-based regimen and (d) reported to be adherence. A study participant was excluded if: (a) placed on PI/r-based ART due to adverse events to an NNRTI-based ART or due to comorbidities, (b) transfer out of the study site, (c) lost to follow-up or dead, and (d) data unavailable.

Data collection

Data were collected from the medical records of each eligible child monitored at the study site and the following variables were abstracted: socio-demographic data (age, gender, level of education, family/guardian); clinical data (ART regimen, duration of ART, WHO clinical staging, CD4 T lymphocyte cells count, viral load measurement at initiation of PI/r-based ART and at the last monitoring); see mini data set attached as supporting file, S1 Table.

Data analysis

Collected data were entered into an electronic datasheet developed using Epi DATA software and analysis was done with the STATA version 3.1 and Microsoft Excel 2010. Statistical significance was set at 5%, using 95% confidence interval (CI). The predictive factors for clinical, immunological and virological conditions were identified in univariate and multivariate analysis using logistic regression at a significance threshold of 5%. Following the definition of therapeutic response, clinical success was defined as WHO stage I or II at the moment of the study; immunological success was defined as CD4 >500 cells/mm³; and viral suppression was defined as HIV-1 RNA <1000 RNA copies/ml.

Ethical considerations

Ethical clearance for the study was obtained from the Institutional review board (IRB) of the Essos Hospital Centre under the reference number 2017/22/CE-CHE; the Hospital Directorate provided an administrative authorization; as per approval from the IRB on the consent procedure, a written proxy-informed consent was obtained from the respective parent/caregiver; a verbal assent was obtained from the child as he/she grew-up; and all data were processed under strict confidentiality and privacy by using unique identifiers.
Results

Characteristics of the study population

Out of 108 CLHIV in the cohort of those receiving PI/r-based ART, 37 were excluded following documented reports of switched to PI/r-based ART due to adverse events or comorbidities, transferred out, lost to follow-up or deaths; giving a total of 71 participants eligible for analysis (Fig 1).

Of the 71 eligible participants, 59.2% were male and 2/3 of them were older than 10 years (i.e. adolescents). Up to 30% were orphans of at least one parent, with the highest proportion of orphans (41.4%) found amongst those on second-line PI/r-based ART (Table 1).

Mean age of children at the moment of ART initiation was 7.1 months for those on first-line PI/r-based ART and 68.3 months for those on second-line PI/r-based ART. At the moment of the study, the mean age for children on a first- and second-line PI/r-based ART was 5.5 years and 13.4 years respectively. The median duration of ART was 84 months overall.

Clinical, immunological and virological responses to ART

In the entire study population, the clinical success was observed to be 100% (71/71) in both groups. Of note, majority of children were at the WHO stage I (94.4%) while the remaining fewer cases (5.6%) were classified as WHO II. Following immunological success, 95.2% had a CD4 count greater than 500 cells/μl. following viral suppression, 74.7% had a plasma viral load below 1000 RNA copies/mL. Of note, viral suppression was slightly higher amongst children

Fig 1. Flow chart for enrolment of the study participants.

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on a first-line (76.2%) versus those on second-line PI/r-based regimen (72.4%); as shown in Table 2.

Factors associated to therapeutic success (univariate and multivariate analysis). As substantial difference in response was found mainly with viral load, associated factors were then evaluated with respect to this variable. It appeared that, the child age at the moment of the study, the educational level, living with their parents/guardians, were independent factors of viral suppression (viral load \( <1000 \) RNA copies/mL). Particularly, older children were less likely to experience viral suppression. Children aged 15–18 years were nine times less likely to achieve viral suppression compared to those aged \( \leq 10 \) years (Odd Ratio = 0.08); similarly, children aged 10–14 years were eight times less likely to achieve viral suppression compared to those aged \( \leq 10 \) years (Odd Ratio = 0.22), as shown in Tables 3 and 4.

Discussion

With paucity of data on response to paediatric PI/r-based ART and their determinants in SSA, generating evidence on clinical, immunological and virological responses would serve in designing strategies with maximal outcomes in these settings.

As primary outcomes, clinical and immunological status appear satisfactory, while viral suppression on PI/r-based ART was considered suboptimal (75%) among these children, especially regarding the duration on ART (median: about seven years). Therefore, even in SSA settings, our findings support the use of viral load in routine monitoring in order to detect ART failure on time [8]. Interestingly, the similar rate of viral suppression first and second-line PI/r-based ART-experiencing children suggest comparable risk of ART failure in both ART lines in these settings [12–15].
Based on the high rate of virological failure on PI/r-containing regimens (especially for those on second-line with a viral load above 1000 RNA copies/mL), it appears urgent to question the introduction of third-line of ART using innovative molecules such as integrase strand-transfer inhibitors in pediatric care [11]. Such new drugs might help CLHIV failing second-line PI/r-based ART to achieve viral re-suppression and regain a normal life span [16]. Our results are similar to data from Uganda (15% virological failure after 48 months) and from Thailand (81% of children with viral suppression after 48 weeks of second line ART) [13,14]. Nonetheless, our rate of virological failure on second-line PI/r-based ART appears more favourable (23.8%) compared to reports from Bangui among similar children (47% virological failure after 18–30 months) [12]. A possible reason could be in the difference in monitoring between the countries [16]. Also, resistance profile is unknown in the aforementioned studies, which in turn require further investigations [16–18].

Regarding the median time on second line ART (7 years), we could there postulate that at least 20% of our children are on a failing regimen once they are treated for a similar period of time. As second-line in paediatric remains the ultimate ART-line in our context, an appeal for access to HIVDR testing appear as a key weapon in combating risk of multi-resistance by sequencing the most potentially active ingredients, as reported in South-African children [15].

As viral suppression in children appears to be associated with younger age (<10 years old), living with parents, being on first-line PI/r-based ART regimens and having a low educational level, it would be of great clinical importance to design a closer or specific monitoring strategy once a child enters adolescence, for those on a long-term second-line regimen. Such corrective measures would improve adherence and sustain viral suppression [22].

Our findings would have provided stronger evidence with adequate measurements of adherence (self reported in at study site) and with HIVDR profiling to confirm ART failure or to suspect non-adherence/poor bioavailability. The threshold used for viral suppression suggests on-going replication and risk for selecting resistant variants for children with low-level viremia (viral load between 40–999 RNA copies/mL) [23–24].

**Table 2. Therapeutic outcomes of children on protease inhibitor-based antiretroviral therapy.**

| Variables                        | Substitute PI based ART | Initial PI based treatment | Total |
|----------------------------------|-------------------------|---------------------------|-------|
|                                  | Number (n = 29) Frequency (%) | Number (n = 42) Frequency (%) | Number (n = 71) Frequency (%) |
| WHO clinical stage               | WHO I 25 86.2 | 42 100 | 67 94.4 |
|                                  | WHO II 4 13.8 | 0 0 | 4 5.6 |
|                                  | WHO III 0 0 | 0 0 | 0 0 |
|                                  | WHO IV 0 0 | 0 0 | 0 0 |
| Absolute CD4 cell count          | <350 1 4.2 | 0 0 | 1 1.7 |
|                                  | 350–499 1 4.2 | 1 2.8 | 2 3.3 |
|                                  | > 500 22 91.6 | 35 97.2 | 57 95 |
| Viral load (copies/ml)           | <1000 21 72.4 | 32 76.2 | 53 74.7 |
|                                  | ≥1000 8 27.6 | 10 23.8 | 18 25.3 |

ART: antiretroviral therapy.

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### Table 3. Factors associated with viral suppression in univariate analysis.

| Independent variables                      | Viral suppression (%) | Khi² p value at 5% |
|--------------------------------------------|-----------------------|-------------------|
|                                            | Yes | No  |                              |
| **Current age of the child (years)**       |     |     |                              |
| 2–9                                       | 100 | 0   | 0.000⁺                        |
| 10–14                                     | 63.6| 36.4|                              |
| 15–18                                     | 25  | 75  |                              |
| **Level of education**                     |     |     | 0.000⁺                        |
| None                                      | 100 | 0   |                              |
| Primary                                   | 100 | 0   |                              |
| Secondary                                 | 36.4| 63.6|                              |
| **Line of treatment**                      |     |     | 0.719                         |
| Initial PI/r-based ART                    | 76.2| 23.8|                              |
| Second-line PI/r-based ART                | 72.4| 27.6|                              |
| **Living with parents or guardians**       |     |     | 0.049⁺                        |
| Parent                                    | 87.9| 12.1|                              |
| Guardian                                  | 57.1| 42.9|                              |
| **Gender**                                |     |     | 0.592                         |
| Male                                      | 81.5| 18.5|                              |
| Female                                    | 84.6| 15.4|                              |
| **Orphan or not**                         |     |     | 0.631                         |
| Orphan                                    | 78.6| 21.4|                              |
| Non orphan                                | 84.6| 15.4|                              |

ART: antiretroviral therapy; PI/r: ritonavir boosted protease inhibitor; viral suppression is defined as plasma viral load < 1000 copies/mL.

⁺Significant

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### Table 4. Factors associated to viral suppression in multivariate analysis.

| Independent variables                      | P value | Odd Ratio | 95% confidence Interval |
|--------------------------------------------|---------|-----------|-------------------------|
| **Current age of the child (years)**       |         |           |                         |
| 2–9 (ref)                                  | -       | -         | -                      |
| 10–14                                     | 0.000⁺  | 0.22      | [0.07 ; 0.73]           |
| 15–18                                     | 0.000⁺  | 0.08      | [0.02 ; 0.57]           |
| **Level of education**                     |         |           |                         |
| None (ref)                                 | -       | -         | -                      |
| Primary                                   | 0.095   | 7.98      | [0.73 ; 23.5]           |
| Secondary                                 | 0.372   | 0.57      | [0.17 ; 1.95]           |
| **Living with parents or guardians**       |         |           |                         |
| Parent (ref)                               | -       | -         | -                      |
| Guardian                                  | 0.069   | 0.18      | [0.03 ; 1.14]           |
| **Treatment regimen**                      |         |           |                         |
| Initial PI based treatment (ref)           | -       | -         | -                      |
| Second-line PI based ART                  | 0.648   | 0.55      | [0.04 ; 6.68]           |

ART: antiretroviral therapy; Ref: Reference; PI/r: ritonavir boosted protease inhibitor; viral suppression is defined as viral load < 1000 copies/mL.

⁺Significant

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Another limitation to this study is the fact that children with higher age might also reflect a longer ART exposure. Of note, in the absence of data on adherence and resistance, the poorer virological outcome observed among adolescents could not be attributed solely to a worse adherence, but also to their potential longer duration on ART as compared to younger children. This therefore calls for more emphasis on the need of viral load in monitoring ART, and timely resistance test and adherence assessment in case of virological failure.

Conclusion

After seven years of PI/r-based ART experience, CLHIV in a typical urban setting of Cameroon have favourable clinical and immunological outcomes. However, the poor rate of viral suppression (<80%) requires interventions in the clinical settings mainly towards adolescents and those on a second-line PI-based regimen.

Supporting information

S1 Table. Mini data set of children enrolled on protease inhibitor treatment as Essos Hospital Centre. ART: antiretroviral therapy, NNRTI: non-nucleoside reverse transcriptase inhibitor; PI/r: ritonavir boosted protease inhibitor.

(XLS)

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References

1. UNAIDS report on the global AIDS epidemic 2016 http://www.WhoInthivpubmeunaidsglobalreporten.
2. Seven stocktaking report UNICEF, 2016
3. Newell M-L, Coovadia H, Cortina-Borja M, Rollins N, Gaillard P, Dabis F, et al. Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. The Lancet. 2004; 364 (9441): 1236–1243.
4. WHO consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, WHO, Geneva 2013
5. World Health Organization. (2016). Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. World Health Organization.
6. Start Free, Stay Free, AIDS Free—A super-fast-track framework for ending AIDS among children, adolescents and young women by 2020, 2016, <http://www.unaids.org/sites/default/
7. Davies M-A, Pinto J, Bras M. Getting to 90-90-90 in paediatric HIV: What is needed? Journal of the International AIDS Society. 2015; 18 (7Suppl 6): 20770. https://doi.org/10.7448/IAS.18.7.20770 PMID: 28326130
8. Njom Nlend AE, Motaze AN, Ndiang ST, Fokam J. Predictors of Virologic Failure on First-line Antiretroviral Therapy Among Children in a Referral Pediatric Center in Cameroon. Pediatr Infect Dis J. 2017 Nov; 36(11):1067–1072. https://doi.org/10.1097/INF.0000000000001672 PMID: 28661967
9. Fokam J, Billong SC, Jogue F, Ndiang SMT, Motaze ACN, Paul KN, et al. Immuno-virological response and associated factors amongst HIV-1 vertically infected adolescents in Yaoundé-Cameroon. PloS one. 2017; 12(11), e0187566. https://doi.org/10.1371/journal.pone.0187566 PMID: 29112991
10. Fokam J, Salpini R, Santoro MM, Cento V, Perno CF, Colizzi V, et al. Drug resistance among drug-naive and first-line antiretroviral treatment-failing children in Cameroon. The Pediatric infectious disease journal. 2011; 30(12), 1062–1068 https://doi.org/10.1097/INF.0b013e31822db54c PMID: 21817951
11. Dehority W, Abadi J, Wiznia A, Viani RM. Use of integrase inhibitors in HIV-infected children and adolescents. Drugs. 2015; 75(13), 1483–1497. https://doi.org/10.1007/s40265-015-0446-2 PMID: 26242765
12. Charpentier C, Gody JC, Mbti kon O, Moussa S, Matta M, Pérè H, et al. Virological response and resistance profiles after 18 to 30 months of first-or second-/third-line antiretroviral treatment: a cross-sectional evaluation in HIV type 1-infected children living in the Central African Republic. AIDS research and human retroviruses, 2012; 28(1), 87–94. https://doi.org/10.1089/AID.2011.0035 PMID: 21599597
13. Musiime V, Kaudha E, Kayiwa J, Mirembe G, Odera M, Kizito H, et al. Antiretroviral drug resistance profiles and response to second-line therapy among HIV type 1-infected Ugandan children. AIDS research and human retroviruses. 2013; 29(3), 449–455. https://doi.org/10.1089/aid.2012.0283 PMID: 23308370
14. Puthanakit T, Jourdain G, Suntarattiwong P, Chokephaibulkit K, Siangphoe U, Suwanlerk T, et al. High virologic response rate after second-line boosted protease inhibitor-based antiretroviral therapy regimen in children from a resource limited setting. AIDS research and therapy. 2012; 9(1), 20. https://doi.org/10.1186/1742-6405-9-20 PMID: 22709957
15. Meyers T, Sawry S, Wong JY, Moultrie H, Pinillos F, Fairlie L, et al. Virologic failure among children taking lopinavir/ritonavir-containing first-line antiretroviral therapy in South Africa. The Pediatric infectious disease journal. 2015; 34 (2), 175. https://doi.org/10.1097/INF.0000000000000544 PMID: 25741970
16. Boerma RS, Boender TS, van Hensbroek MB, Rinke de Wit TF, Sigaloff KC. Sequencing paediatric antiretroviral therapy in the context of a public health approach. Journal of the International AIDS Society. 2015; 18 (7S6).
17. Rojas Sánchez P, Prieto L, Jiménez De Orý S, Fernández Cooke E, Navarro ML, Ramos JT, et al. Impact of lopinavir/ritonavir exposure in HIV-1 infected children and adolescents in Madrid, Spain during 2000–2014. PLoS One. 2017; 12(3):e0173168. https://doi.org/10.1371/journal.pone.0173168 PMID: 28350802
18. Zamora L, Gatell JM. Efficacy of initial antiretroviral therapy based on lopinavir/ritonavir plus 2 nucleoside/nucleotide analogs in patients with human immunodeficiency virus type 1 infection. Enferm Infecc Microbiol Clin. 2014; 32 Suppl 3:2–6.
19. Van Dyke RB, Patel K, Kagan RM, Karalius B, Traite S, Meyer WA III, et al. Antiretroviral drug resistance among children and youth in the United States with perinatal HIV. *Clinical Infectious Diseases*. 2016; 63(1), 133–137. https://doi.org/10.1093/cid/ciw213 PMID: 27056398

20. Pursuing Later Treatment Options II (PLATO II) project team for the Collaboration of Observational HIV Epidemiological Research Europe (COHERE. Risk of triple-class virological failure in children with HIV: a retrospective cohort study. *The Lancet*. 2011; 377(9777), 1580–1587.

21. Suaysod R, Ngo-Giang-Huong N, Salvadori N, Cressey TR, Kanjanavanit S, Techakunakorn P, et al. Treatment Failure in HIV-Infected Children on Second-line Protease Inhibitor–Based Antiretroviral Therapy. *Clinical Infectious Diseases*. 2015; 61 (1), 95–101. https://doi.org/10.1093/cid/civ271 PMID: 25838288

22. Weijsenfeld AM, Smit C, Cohen S, Wit FWNM, Mutschelknauss M, van der Knaap LC, et al. Virological and Social Outcomes of HIV-Infected Adolescents and Young Adults in the Netherlands Before and After Transition to Adult Care. *Clin Infect Dis*. 2016; 63 (8):1105–1112. https://doi.org/10.1093/cid/ciw487 PMID: 27439528

23. Siberry GK, Amzel A, Ramos A, Rivadeneira ED. Impact of Human Immunodeficiency Virus Drug Resistance on Treatment of Human Immunodeficiency Virus Infection in Children in Low- and Middle-Income Countries. *J Infect Dis*. 2017; 216 (suppl_9), S838–S842. https://doi.org/10.1093/infdis/jix407 PMID: 29045697

24. Boender TS, Kityo CM, Boerma RS, Hamers RL, Ondoa P, Wellington M, et al. Accumulation of HIV-1 drug resistance after continued virological failure on first-line ART in adults and children in sub-Saharan Africa. *J Antimicrob Chemother*. 2016; 71(10), 2918–2927. https://doi.org/10.1093/jac/dkw218 PMID: 27342546