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Pretreatment HIV drug resistance results in virological failure and accumulation of additional resistance mutations in Ugandan children

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Background: Pretreatment HIV drug resistance (PDR) can impair virological response to ART, jeopardizing effective treatment for children.

Methods: Children aged ≤12 years initiated first-line ART in Uganda during 2010–11. Baseline and 6 monthly viral load (VL) and genotypic resistance testing if VL >1000 copies/mL was done. The 2015 IAS-USA mutation list and Stanford algorithm were used to score drug resistance mutations (DRMs) and susceptibility. Virological failure (VF) was defined as two consecutive VLs >1000 copies/mL or death after 6 months of ART. Factors associated with failure and acquired drug resistance (ADR) were assessed in a logistic regression analysis.

Results: Among 317 children enrolled, median age was 4.9 years and 91.5% received NNRTI-based regimens. PDR was detected in 47/278 (16.9%) children, of whom 22 (7.9%) had resistance against their first-line regimen and were therefore on a partially active regimen. After 24 months of follow-up, 92/287 (32.1%) had experienced VF. Children with PDR had a higher risk of VF (OR 15.25, $P<0.001$) and ADR (OR 3.58, $P=0.01$).

Conclusions: Almost one-third of children experienced VF within 24 months of NNRTI-based first-line treatment. PDR was the strongest predictor of VF and ADR, and therefore presents a major threat in children. There is a need for ART regimens that maximize effectiveness of first-line therapy for long-term treatment success in the presence of PDR or incorporation of routine VL testing to detect VF and change treatment in time, in order to prevent clinical deterioration and accumulation of additional drug resistance. Children ≤3 years should be initiated on a PI-based regimen as per WHO guidelines.

Introduction

Sub-Saharan Africa has the highest burden of HIV-infected children in the world.[1] There has been unprecedented acceleration of access to ART in the last 10 years in low- and middle-income countries (LMIC). By the end of 2015, an estimated 823000 HIV-infected children were receiving ART in LMIC,[1] the large majority on first-line regimens. In Uganda, of the estimated 95649 children living with HIV, 60029 were accessing ART at the end of 2015.[2]

Although access to ART has conferred substantial benefits on survival and quality of life, it has also caused the emergence of both acquired[3–6] and transmitted drug resistance, especially in eastern and southern Africa.[7–10] As most infected children have acquired HIV from their mothers, they are particularly at risk of HIV drug resistance in the context of prevention of mother-to-child transmission (PMTCT) or via transmission of resistant strains from their mothers.[11] Our group has previously shown high rates of HIV drug resistance among children initiating ART in Uganda.[12]

Pretreatment drug resistance (PDR) has the potential to contribute to increasing rates of virological failure (VF) at a population level, thus compromising the long-term effectiveness of recommended first-line regimens.[13] Treatment failure is more frequent among children and adolescents compared with adults and this translates into a higher risk of developing acquired drug resistance (ADR).[14,15] Studies in sub-Saharan Africa have shown that VF rates on NNRTI-based first-line ART were significantly higher in children compared with adults.[16] In another study from Côte d'Ivoire, only 66% of children achieved virological success after a median of 10.2 months of ART. Among those children experiencing VF, 71% of viruses displayed resistance to at least one antiretroviral drug.[17]

In view of the fact that HIV drug resistance is a growing problem, and given the fact that children are especially vulnerable, this study seeks to describe virological outcomes of children on first-line treatment, and to evaluate the effect of PDR and other factors on treatment failure and accumulation of drug resistance mutations (DRMs). The results will provide important information to develop appropriate interventions aimed at enhancing the effectiveness of currently available first-line regimens.

Methods

Study design and setting

The Monitoring Antiretroviral Resistance in Children (MARCH) study is a multicentre prospective observational cohort study of HIV-1-infected children who received HIV treatment and care at three Joint Clinical Research Center (JCRC) Regional Centers of Excellence (RCEs) based in Kampala, Mbale and Fort Portal, Uganda.

Ethics

The ethics committees of JCRC (approval reference 30 October 2009) and the Uganda National Council of Science and Technology (approval reference HS 721), and the Academic Medical Center of the University of Amsterdam in the Netherlands, approved the study protocol before commencement of the study (approval reference 09.17.1626).

Study participants

Study methods of this cohort have previously been described in detail.[12] We enrolled HIV-infected children aged ≤12 years from January 2010 to August 2011. For this analysis, participants starting on first-line ART were included and followed up for 24 months.

Children were started on ART based on 2006 WHO treatment guidelines,[18] though by August 2010 the clinics had adopted the revised treatment guidelines for 2010.[19] Following the revision, all children <24 months of age were eligible for ART irrespective of their clinical stage or CD4 cell counts. A combination of two NRTI drugs and one NNRTI (either efavirenz or nevirapine) were the preferred combinations of choice and recommended by the Ugandan guidelines.[20] PI-based regimens were prescribed for young children exposed to PMTCT. Efavirenz was only given to children >3 years.

Follow-up procedures and variables measured

Sociodemographic and clinical data were collected at enrolment and at subsequent 3 monthly follow-up visits and aggregated in a web-based database. At baseline and every 6 months thereafter, viral load (VL) testing was done as well as genotypic resistance testing on specimens with VL >1000 copies/mL. Major drug resistance mutations were identified based on the 2015 IAS mutation list.[21] Susceptibility to the prescribed regimen was determined by calculating the genotypic sensitivity score (GSS) using the Stanford algorithm (version 7.0).[22] Reduced susceptibility to the prescribed regimen was defined as GSS <3, i.e. <3 fully susceptible drugs. Thirty day adherence based on missing doses in the last 30 days was based on caregiver's report; adherence over time was calculated as the mean of these adherence reports, and was categorized as being suboptimal (≤95% adherence) or optimal (>95% adherence). VF was defined according to the WHO as two consecutive detectable VL >1000 copies/mL taken at least 6 months after treatment initiation.[23] A VL >1000 copies/mL at the last available measurement, or death after at least 6 months of treatment, was also considered as failure. Children were excluded from the analysis if they had fewer than two VL measurements during follow-up. ADR was defined as a new DRM following initiation of ART for both children with or without PDR.

GenBank accession numbers

All HIV-1 *pol* sequences in this study have been deposited in GenBank under the following accession numbers: MF357928–MF358296.

Statistical analysis

Baseline characteristics are presented as proportions or medians with IQR. Group comparisons for categorical data were performed using the χ^2 test or Fisher's exact test, and for continuous data using the *t*-test or the Wilcoxon rank-sum test. Logistic regression was performed to model the association of the presence of PDR with the outcomes VF and ADR. Explanatory variables considered in the analysis were age, sex, WHO clinical stage at study entry, PDR, VL at study initiation, adherence, NNRTI used, exposure of child to drugs for PMTCT and immunodeficiency for age (defined as normal if age <5 years and CD4% ≥25% or if age ≥5 years and CD4 count >500 cells/mm^3; diminished if age <5 years and CD4% 10%–25% or age ≥5 years and CD4 count 100–500 cells/mm^3; and immunodeficient if age <5 years and CD4% <10% or age ≥5 years and CD4 count <100 cells/mm^3). Explanatory variables associated with the outcome variables ($P < 0.10$) in the univariable analysis were included in the multivariable model in a stepwise approach. Biologically plausible interactions were examined. Results were expressed as OR with 95% CI and *P* values, with $P < 0.05$ regarded as statistically significant. Analyses were performed using the statistical software package STATA version 12 (STATA Corp. LP, Texas, TX, USA).

Results

Patient characteristics

Between January 2010 and August 2011, 317 children aged ≤12 years initiating first-line ART were enrolled into the MARCH study in Kampala ($n = 90$), Mbale ($n = 108$) and Fort Portal ($n = 119$) and followed up for 24 months. Baseline characteristics of the children are summarized in Table 1. Of the 317 participants, 290 (91.5%) initiated a NNRTI-based regimen, 12 (3.8%) a PI-based regimen and 15 (4.7%) started a triple-NRTI regimen, because of PMTCT exposure and tuberculosis treatment for the latter two regimens. The choice to start a child on efavirenz or nevirapine was not random but was based on age, clinician's preference and availability.

Pretreatment drug resistance

Genotype results before treatment initiation were available for 278 (87.7%) children. At least one DRM was detected in 47 (16.9%) participants; of these children, 22 (7.9%) had predicted reduced susceptibility to at least one drug of their first-line regimen (Table 1). Among participants with drug resistance who initiated a fully active regimen, the E138A mutation, which confers resistance to the second-generation NNRTI rilpivirine, was detected in 18/25 (72%) children.

Table 1. Baseline characteristics of a cohort of 317 HIV-infected children initiating first-line ART at three clinics in Uganda (January 2010–August 2011)

	Summary statistics		
Variable	number or median (IQR)	cohort	percentage
Age <3 years	113	317	35.6
Sex (male)	159	317	50.2
Study site			
Kampala	90	317	28.4
Fort Portal	119	317	37.5
Mbale	108	317	34.1
WHO stage			
1	25	317	7.9
2	62	317	19.6
3	162	317	51.1
4	68	317	21.5
Height for age *z* score < −2	155	302	51.3
Weight for age *z* score < −2	97	266	36.5
CD4% (in children <5 years)	19 (12–29)	159	
CD4 count (in children ≥5 years)	350 (223–689)	146	
Pretreatment drug resistance[a]			
no	231	278	83.1
yes, but on fully active regimen	25	278	9.0
yes, on partially active regimen	22	278	7.9
Log viral load at baseline	5.1 (4.5–5.6)	309	
Subtype			
A	153	286	53.5
D	84	286	29.4
C/G	10	286	3.5
CRF	23	286	8.0
URF	16	286	5.6
PMTCT exposed			
yes	16	317	5.0
no	266	317	83.9
unknown	35	317	11.0
Initial ART regimen			
NNRTI-based	290	317	91.5
triple NRTI	15	317	4.7
PI-based	12	317	3.8
Primary caregiver			
mother	178	317	56.2
father	18	317	5.7
other	121	317	38.2
primary school or higher	247	317	77.9
Parental status			
both alive	152	255	59.6
both deceased	39	255	15.3
Immunological status			
normal[b]	109	305	35.7
diminished[c]	154	305	50.5
immunodeficient[d]	42	305	13.8

CRF, circulating recombinant form; URF, unique recombinant form.
[a]Based on genotypic sensitivity score.
[b]Age <5 years and CD4% ≥25% or age ≥5 years and CD4 count >500 cells/mm^3.
[c]Age <5 years and CD4% 10%–25% or age ≥5 years and CD4 count 100–500 cells/mm^3.
[d]Age <5 years and CD4% <10% or age ≥5 years and CD4 count <100 cells/mm^3.

Follow-up

At 12 and 24 month follow-up a total of 287 (90.5%) and 261 (82.3%) participants, respectively, were still on first-line ART and in care. An additional seven participants were still in care but had switched to second-line therapy by 24 months. The participants who did not complete follow-up included 13 (4.1%) who died (8 in the first 6 months), 12 (3.8%) who were lost to follow-up and 24 (7.6%) who transferred out of the recruiting ART centres (Figure 1).

Virological response

VL at the 6, 12, 18 and 24 month visits was assessed for 81.3% (239/294), 90.2% (259/287), 89.1% (246/276) and 96.2% (251/261), respectively, of the participants who were still receiving first-line therapy. Virological suppression (VL <1000 copies/mL) was achieved by 80.3% (191/239), 77.0% (197/256), 76.8% (189/246), and 71.3% (179/251) at months 6, 12, 18 and 24, respectively (Figure 1).

Overall, 92/287 (32.1%) children experienced VF during 24 months of follow-up, of which 1 (1.1%) was on PI-based treatment. Thirty children (10.5%) had an unknown status of VF because they had fewer than two VL results, due to transfer out, loss to follow-up or death before 6 months of treatment. Compared with those with known status of VF, these participants were younger (2.1 versus 4.9 years; $P = 0.012$) and initiated triple nucleoside therapy more frequently (16.7% versus 3.5%; $P = 0.008$). The proportion of children with PDR was not significantly different in both groups.

In a multivariable analysis, children with PDR were more likely to experience VF [adjusted odds ratio (aOR) 15.25, 95% CI 3.77–61.67; $P < 0.001$; Table 2]. Other predictors associated with VF included higher baseline VL (aOR 2.28 for every log VL increase, 95% CI 1.57–3.31; $P < 0.001$) and WHO clinical stage 2 compared with stage 1 (aOR 10.395% CI 1.41–75.56, $P = 0.022$, Table 2). None of the other baseline characteristics, including age, sex, CD4%/CD4 count, HIV subtype and type of NNRTI, was predictive of failure.

Acquired drug resistance (ADR)

Sequence results were available for 39/48 (81.3%), 51/62 (82.3%), 47/57 (82.5%) and 61/72 (84.7%) children with a VL >1000 copies/mL at months 6, 12, 18 and 24, respectively (Figure 1). Of 278 children with Genotypic Resistance Test (GRT) results during follow-up, 72 children (25.9%) acquired additional DRMs, 68 (24.5%) acquired additional NNRTI mutations and 67 (24.1%) acquired additional NRTI mutations. In children who met the study definition of VF and had GRT results available, 67/84 (79.8%) acquired additional DRMs, 64 (76.2%) acquired NNRTI mutations and 63 (75.0%) NRTI mutations. No PI mutations were detected (Figure 2). There were 63 children with acquired dual-class drug resistance, 5 with only NNRTI resistance and 4 with only NRTI resistance.

Table 3 shows predictors of ADR in the multivariable model. Children with PDR had significantly higher odds of developing ADR (aOR 3.58, 95% CI 1.35– 9.51; $P = 0.010$). The other predictors for ADR included higher VL before treatment initiation (aOR 2.16, 95%

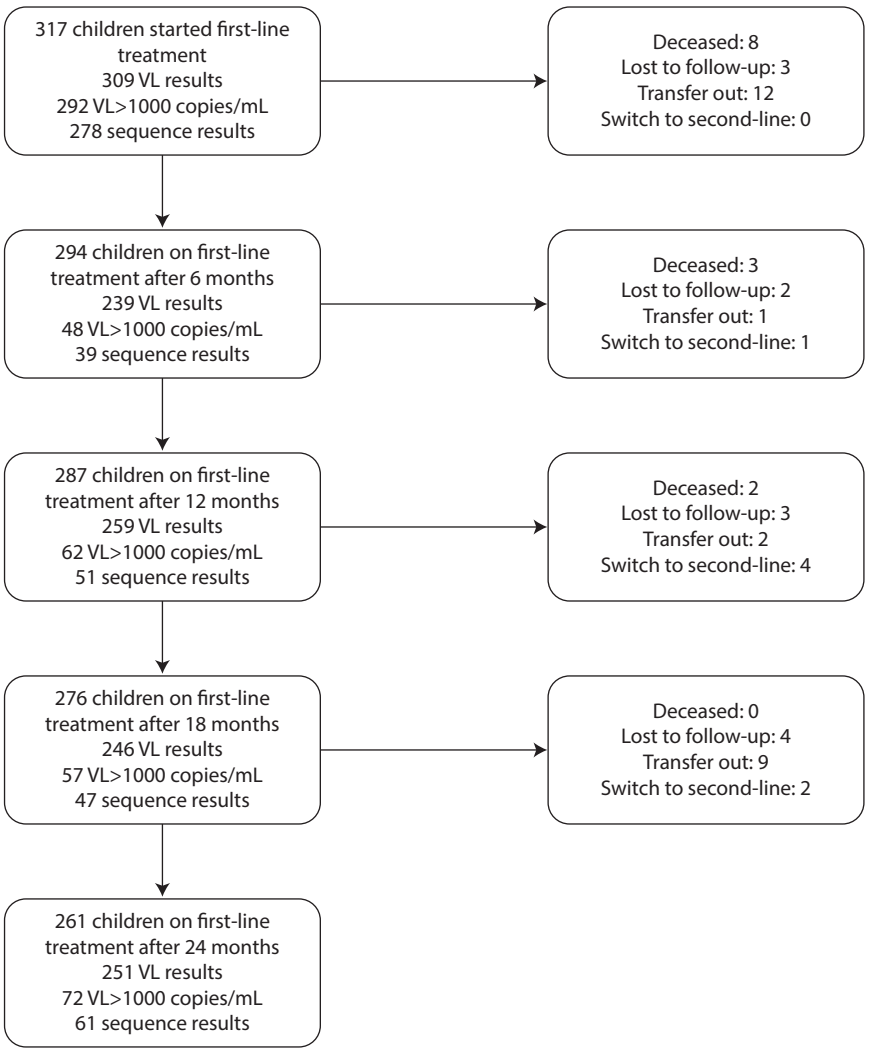

Figure 1. Follow-up of children in this cohort. VL, viral load.

CI 1.46–3.22; $P < 0.001$) and WHO stage 2 compared with stage 1 (aOR 10.14, 95% CI 1.12–91.71; $P = 0.039$).

Discussion

This study of 317 ART-naive Ugandan HIV-infected children evaluated the effect of PDR and other factors on virological outcomes and on developing ADR. PDR was common as it was observed in one out of every six ART-naive children starting ART. This has important consequences, as a third of all children failed on first-line ART within 2 years and PDR was the most important predictor for this failure. Children with PDR were more than 15 times as likely to experience VF compared with children who received a fully active regimen. In addition, children with PDR were three times more likely to acquire additional DRMs. Other factors significantly associated with VF and ADR included baseline VL and WHO stage 2 compared with stage 1. Adherence was not associated with these ART outcomes. In univariate analysis, immune-deficient status and use of NNRTI nevirapine compared with efavirenz were associated with treatment failure and ADR while HIV subtype, PMTCT exposure age and sex were not associated.

The effects of PDR on treatment outcomes observed in this study confirm results mainly from adult cohorts on treatment for 6–18 months in developed countries[24–29] and developing countries.[13,30–32] The results also indicate that the effect of PDR on treatment outcomes might be even stronger in children than in adults as we found the OR for failure was 15.3 in children with PDR, while a large cohort study in adults previously conducted by our research group showed an OR of 2.1. The OR for ADR was 3.6 in our study, compared with 2.3 in adults.[13]

Selecting an initial regimen has longstanding consequences for future therapy. In the context of a public health approach, the WHO ART guidelines recommend ritonavir-boosted lopinavir-based regimens for all children ≤3 years of age irrespective of PMTCT exposure.[23] This recommendation is based on evidence of the superiority of a lopinavir/ritonavir-based regimen for infants and young children in terms of efficacy, safety and

Table 2. Factors associated with virological failure among children in this cohort

		Univariable			Multivariable		
Variable	VF (*n*/*N*)	OR	95% CI	*P*	OR	95% CI	*P*
Age							
≥3 years	59/192	1					
<3years	33/95	1.2	0.71–2.02	0.494			
Sex							
male	53/145	1					
female	39/142	0.66	0.40–1.08	0.100			
WHO stage at treatment initiation							
1	2/24	1			1		
2	22/57	6.91	1.48–32.34	0.014	10.3	1.41–75.56	0.022
3	43/148	4.50	1.01–20.0	0.048	3.5	0.49–24.92	0.212
4	25/56	8.33	1.79–38.79	0.007	3.8	0.51–28.82	0.191
Activity of first-line regimen[a]							
fully active	69/240	1			1		
partially active	15/20	8.9	2.9–27.8	<0.001	15.25	3.77–61.67	<0.001
Baseline VL (log copies/mL)		1.82	1.35–2.45	<0.001	2.28	1.57–3.31	<0.001
Adherence							
>95%	65/215	1			1		
≤95%	27/72	1.38	0.79–2.42	0.254	1.97	0.95–4.12	0.070
NNRTI used							
nevirapine	61/164	1					
efavirenz	25/104	0.53	0.31–0.92	0.023			
PMTCT exposed							
yes	4/15	1					
no	80/241	1.37	0.42–4.43	0.603			
Immunological status							
normal[b]	29/106	1					
diminished[c]	44/137	1.26	0.72–2.19	0.423			
immunodeficient[d]	16/34	2.36	1.06–5.24	0.035			
Subtype							
A	42/138	1					
D	29/79	1.33	0.74–2.38	0.344			
C/G	3/10	0.98	0.24–3.97	0.977			
CRF	6/21	0.91	0.33–2.52	0.862			
URF	4/12	1.14	0.33–4.00	0.835			

Analysis is corrected for study site (Kampala, Fort Portal or Mbale). PMTCT, prevention of mother-to-child transmission; VF, virological failure; VL, viral load.

[a]Based on genotypic sensitivity score.

[b]Age <5 years and CD4% ≥25% or age ≥5 years and CD4 count >500 cells/mm^3.

[c]Age <5 years and CD4% 10%–25% or age ≥5 years and CD4 count 100–500 cells/mm^3.

[d]Age <5 years and CD4% <10% or age ≥5 years and CD4 count <100 cells/mm^3.

robustness of PI-based regimens in terms of developing PI mutations and thymidine analogue mutations (TAMs)[33–35] and studies that have demonstrated compromised response to nevirapine-containing first-line ART in children exposed to NNRTI used for PMTCT.[36,37] Current Ugandan guidelines,[38] however, still recommend NNRTI-based regimens as the preferred first-line treatment for PMTCT-unexposed children. In Uganda and other developing countries where genotypic testing is not routinely available or feasible, first-line regimens containing boosted-PI should be implemented for all children ≤3 years as recommended by WHO.[23]

In our study, only 12 children started a PI-based first-line regimen, of whom 1 experienced treatment failure. It was not possible to assess the association of drug class with VF or ADR, because the number of children that started PI-based ART was too small. However, given that the presence of NRTI/NNRTI-associated mutations resulting in a partially active first-line regimen was associated with NNRTI-based first-line failure, it is expected that a PI-based ART might have prevented treatment failure. We have shown in studies of children[39] and adults[40] that suppression of resistant virus (with NRTI and NNRTI mutations) is still possible using a PI-based regimen. Current WHO guidelines only recommend

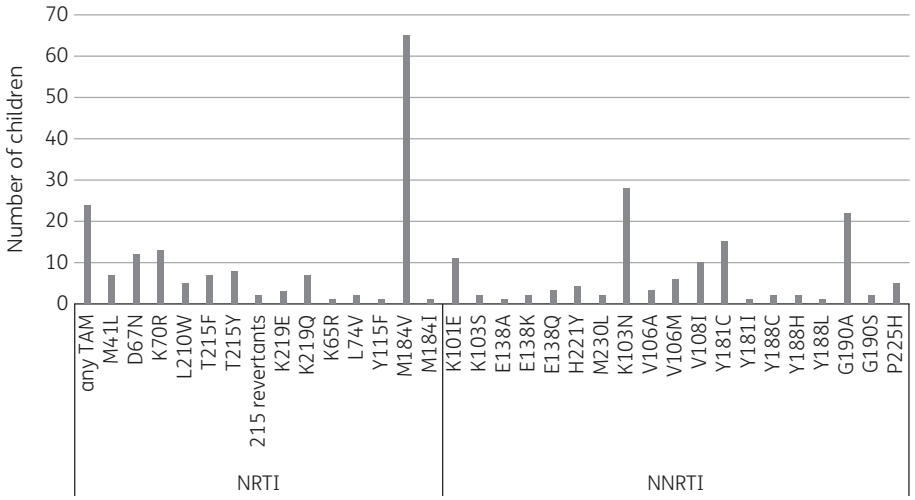

Figure 2. Number of children acquiring mutations on first-line antiretroviral treatment in this cohort. No PI mutations were detected. TAM, thymidine analogue mutations.

Table 3. Factors associated with acquired drug resistance among children in this cohort

		Univariable			Multivariable		
	ADR (*n*/*N*)	OR	95% CI	*P*	OR	95% CI	*P*
Age							
≥3 years	48/183	1					
<3 years	24/95	0.95	0.54–1.68	0.862			
Sex							
male	43/140	1					
female	29/138	0.60	0.35–1.03	0.066			
WHO stage at baseline							
1	1/20	1			1		
2	17/59	7.69	0.95–62.07	0.056	10.14	1.12–91.71	0.039
3	35/145	6.05	0.78–46.80	0.085	6.37	0.73–55.90	0.095
4	19/54	10.31	1.28–83.14	0.028	6.19	0.68–56.68	0.107
Activity of first-line regimen[a]							
fully active	61/256	1			1		
partially active	11/22	3.20	1.32–7.74	0.010	3.58	1.35–9.51	0.010
Baseline VL (log)		1.77	1.25–2.52	0.001	2.16	1.46–3.22	<0.001
Adherence							
>95%	54/208	1			1		
≤95%	18/70	0.99	0.53–1.83	0.967	1.46	0.69–3.07	0.325
NNRTI used							
nevirapine	47/148	1					
efavirenz	19/105	0.47	0.26–0.87	0.016			
PMTCT exposed							
yes	2/14	1					
no	64/232	2.29	0.50–10.50	0.288			
Subtype							
A	36/149	1					
D	24/82	1299	0.71–2.38	0.398			
C/G	3/10	1345	0.33–5.48	0.679			
CRF	4/22	0.698	0.22–2.20	0.538			
URF	5/15	1569	0.50–4.89	0.437			

Analysis is corrected for study site (Kampala, Fort Portal or Mbale). ADR, acquired drug resistance; CRF, circulating recombinant form; PMTCT, prevention of mother-to-child transmission; URF, unique recombinant form; VL, viral load.
[a]Based on calculation of genotypic sensitivity score (GSS).

PI-based first-line ART for children <3 years of age. However, the high rate of PDR and its association with treatment failure that we found in our cohort of older children (median age 4.9 years) suggest that PI-based first-line ART for children >3 years might need to be considered as well. In the absence of access to tests to determine PDR and without the widespread use of alternatives to NNRTI-based first-line ART, children need to be monitored closely using VL to determine treatment failure, to ensure a timely switch to a second-line regimen.

Several factors in this study were not correlated to VF and development of drug resistance. Adherence based on caregivers' reports on missed doses in the last 30 days was not associated with ART treatment outcomes. Despite their widespread use, research provides mixed information on the utility of self-report adherence measures for children living with HIV. Some previous studies have failed to show an association between behavioural measures of ART adherence and VL in HIV-infected paediatric patients, while other studies have found strong associations.[41–46] There may have been overestimation of adherence because of recall bias or bias of providing answers that may be viewed favourably. Although it is obvious that adherence is paramount for achieving and maintaining viral suppression, and prevention of drug resistance, PDR was the strongest predictor of VF, and however adherent a child may be, there would be suboptimal viral suppression.

This longitudinal study in children, the largest to date comparing non-B HIV-1 subtypes, did not find an association between virological response and the development of resistance mutations, similar to another study conducted in Europe.[47] Previous data on this issue have focused on a comparison between sub-type B and non-B viruses. Younger age has been associated with VF in children mostly in cross-sectional and retrospective studies that mainly used clinical and immunological failure as end points.[16,48–50] One study showed higher levels of VF in older children.[51] This prospective study, which is well powered, did not show any evidence of age association with VF.

Several studies have shown that there is a poor correlation between clinical and immunological criteria in identifying children with VF, hence it would be expected that WHO clinical staging would not be a predictor of VF, although this study showed that WHO stage 2 versus stage 1 was a predictor of treatment outcomes. Results from other studies have shown mixed results: some studies[48,51] show no association of WHO stage with VF while some show an association.[52]

The major strength of our study is that this is the largest paediatric prospective cohort study in Africa that has evaluated the effect of PDR on treatment response and ADR. The study participants are representative of children seeking HIV care in treatment programmes from three regions of Uganda. Our study also has potential limitations. First, there was no testing for minority mutations. Most children in this cohort were perinatally infected with HIV, but the median age at study entry was 4.9 years. Therefore, it is possible that we have underestimated the prevalence of PDR as mutations might be archived in older children in the absence of selective drug pressure. The extensive Early Infant Diagnosis (EID) programme and the Test and Treat policy involved treating children at a much younger age compared with this study cohort. Furthermore, data were missing on outcome of virological response and ADR for 30 children (9.5%) because of death in the first 6 months of treatment, transfer out and loss to follow-up. However, this rate is lower than observed in other observational treatment cohorts in sub-Saharan Africa.[53,54] The rate of PDR in the group with missing data was similar to the children with VL data so it is expected that the relationship with failure would not change. Finally, we could not analyse the effect of drug class (NNRTI, PI or triple NRTI) on VF or ADR, because the numbers of children who were not on a NNRTI-based regimen were too small.

In conclusion, in the largest study evaluating the effect of PDR in children in Africa, we found that PDR is common and strongly associated with VF and ADR. In the absence of testing for PDR, routine VL testing is needed to detect children with VF and to ensure a timely switch to second-line treatment, in order to prevent clinical deterioration and accumulation of additional drug resistance. Children ≤3 years old should be initiated on a PI-based regimen as per WHO guidelines.

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Transparency declarations

None to declare.

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