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Original article

Prevalence and determinants of virological failure in HIV-infected children on antiretroviral therapy in rural Cameroon: a cross-sectional study

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Background: In Africa, success of antiretroviral treatment (ART) seems to lag behind in children compared with adults, and high therapeutic failure rates have been reported. We aimed to identify prevalence and determinants of virological failure in HIV-infected children treated under programmatic conditions.

Methods: All patients <18 years on ART presenting to the HIV clinic at the Bamenda Regional Hospital, a secondary referral hospital in rural Cameroon, from September 2010 to August 2011, were enrolled in this cross-sectional study. Clinical data, self-reported adherence, CD4$^+$ T-cell counts and viral load were recorded. Therapeutic drug monitoring was performed on stored plasma samples. Determinants of virological failure were identified using descriptive statistics and logistic regression.

Results: A total of 230 children with a mean age of 8.9 years (SD 3.7) were included. At the time of analysis, the mean duration of HAART was 3.5 years (SD 1.7) and 12% had a CD4$^+$ T-cell count <200 cells/µl. In total, 53% of children experienced virological failure (>200 copies/ml). Among children on nevirapine (NVP), plasma levels were subtherapeutic in 14.2% and supratherapeutic in 42.2%. Determinants of virological failure included male sex, lower CD4$^+$ T-cell counts, subtherapeutic drug levels, longer time on ART and a deceased mother. Poor adherence was associated with subtherapeutic NVP plasma levels and advanced disease stages (WHO stage 3/4).

Conclusions: This study demonstrates high virological failure rates and a high variability of NVP plasma levels among HIV-infected children in a routine ART programme in rural Cameroon. Strategies to improve adherence to ART in HIV-infected children are urgently needed.

Introduction

Roll-out of antiretroviral therapy (ART) in Africa has led to a decrease of HIV morbidity and to an increase of life expectancy of infected adults and children [1,2]. However, significant challenges for successful implementation and monitoring of treatment programmes for the estimated 2.3 million children in sub-Sahara Africa remain [3,4]. It is currently estimated that only 28% of HIV-infected children qualifying for HIV treatment actually receive ART [5].

In general, good treatment outcomes and retention in care have been reported from ART programmes in sub-Saharan Africa, although most data are concerning adult populations [6,7]. In children, low programme attrition is frequent and virological outcomes may be less favourable [8–10]. Previous studies suggest response rates varying from only 33–83% in African children after 2 years on ART [11–13]. However, reports from Central Africa are scarce and the number of children followed

for longer periods is limited in these studies [10]. This has precluded the systematic assessment of determinants for virological failure thus far.

Incomplete adherence has been identified as a major cause of drug resistance and treatment failure [14]. Suboptimal adherence in African children seems to be common [15], and drug resistance in children failing on therapy is occurring frequently [13,16,17]. A medication-taking routine, together with the support of caregivers, seems to be pivotal for good adherence and successful ART [18,19].

In the present study, we aimed to assess the rate of virological failure in children in a routine HIV programme in rural Cameroon. We, furthermore, aimed to assess determinants of virological failure and evaluate the role of self-reported adherence in clinical practice, which we validated by measuring plasma drug levels.

Methods

Setting

The paediatric department at the Bamenda Regional Hospital (BRH) in the North-West region of Cameroon provides service to HIV-infected children in the region. The BRH is the largest secondary referral centre in the region with a catchment area of >2 million people. Eligibility for ART is assessed by a therapeutic committee in accordance with the National HIV Program and current WHO guidelines [20] after opportunistic diseases are ruled out or appropriately treated. Adherence counselling is provided to all patients prior to initiation of ART and, if needed, any time thereafter by a team of social workers. At present, ART containing two nucleoside reverse transcriptase inhibitors and nevirapine (NVP) is started in all children <2 years irrespective of $CD4^+$ T-cell count and thereafter according to absolute $CD4^+$ T-cell count level [20]. Ritonavir-boosted lopinavir (LPV/r) is used when children were recently exposed to NVP in prevention of mother-to-child transmission regimens or in second-line regimens. Efavirenz (EFV) is used in case of non-tolerance of NVP. The National HIV Program foresees a 6-monthly follow-up, including full blood count, liver function test and $CD4^+$ T-cell count. Clinical monitoring by a trained physician every 6 months is recommended. Viral load (VL) monitoring is currently not routinely performed. ART commonly used in children includes Triomune (a fixed-dose combination [FDC] of stavudine, lamivudine and NVP), Zidovex (FDC of zidovudine, lamivudine and NVP), Duovir (FDC of zidovudine and lamivudine) and Stocrin (EFV). Liquid formulations are available for zidovudine (Zidovir), lamivudine (Lamivir) and boosted LPV/r (Aluvia). All children receive dose prescriptions following the WHO guidelines [20].

We performed a cross-sectional study by selecting a random sample of HIV-1-infected paediatric patients on ART at the HIV service at the Bamenda Regional Hospital. All children who attended for routine follow-up and drug refill from September 2010 to August 2011 were eligible and included after informed consent was obtained by parents or legal guardians. Sociodemographic data, including age, sex and family status, as well as treatment-related data, including reported adherence, were recorded by a trained study nurse and transferred into an EpiInfo Database (Centers for Disease Control and Prevention, Atlanta, GA, USA). Ethical approval for the study was granted by the National Ethics Committee in Yaounde, Cameroon.

Adherence reported by the child or a caregiver was categorized according to the number of full daily doses taken in the previous 28 days (ART taken on all days [>95% adherence], ART taken on 21–27 days [75–95% adherence] and ART taken on <21 days [<75% adherence]) [14]. Duration, type and dose of currently prescribed and administered ART were extracted from medical files and pharmacy records and included in the database. Children were examined by a physician and clinical stage, complaints and symptoms were recorded. Basic anthropometric measurements, including weight and height, were also recorded. $CD4^+$ T-cell counts and liver functions tests were performed as mandated by the National Program. Additional plasma samples were drawn and stored at -80° and later shipped to the Institute of Virology, University of Bonn (Bonn, Germany) for VL testing (HIV-1 real-time PCR; Abbott laboratories, Abbott park, IL, USA). Virological failure was defined as HIV-1 VL>200 copies/ml according to recent guidelines [21].

Drug concentration measurements

Plasma drug levels of NVP, EFV and LPV/r were measured at the Department of Pharmacy, Radboud University Nijmegen Medical Centre (Nijmegen, the Netherlands) using three validated HPLC assays with ultraviolet detection [22–24]. NVP plasma concentrations <3.0 mg/l were defined as subtherapeutic and >8.0 mg/l as supratherapeutic [25]. EFV plasma concentrations were defined as subtherapeutic at <1.0 mg/l [26]. LPV/r plasma concentrations were defined as subtherapeutic at <1.0 mg/l [27]. Samples were taken on a population basis at a maximum of 20 h after drug ingestion.

Statistical considerations

Characteristics of patients with and without virological failure and with and without stored plasma samples for the analysis of drug levels were compared using unpaired Student's *t*-tests for continuous data and χ^2 tests for categorical data. Analysis of variance was used to compare characteristics of patients according to different

groups of NVP plasma concentrations. Linear regression was used to assess the correlation time on ART and age. Univariate logistic regression was used to analyse determinants of virological failure and poor (<75%) adherence. A multivariable model was established by successive backward elimination and forward addition of the variables with the highest *P*-value and refitting the model with the remaining variables. This procedure was repeated until the final model was obtained including only variables with a $P<0.05$. Sex was selected to remain in the analyses. A significant interaction between age and sex was observed and was introduced into the final multivariable model for the end point virological failure. A sensitivity analysis stratified by age <10 years and ≥10 years was performed. All tests were two-sided and a $P<0.05$ was considered significant. STATA version 12 (STATA Corp, College Station, TX, USA) was used for all analyses.

Results

At the start of the study in September 2010 a total of 328 HIV-infected children were registered in the ART programme of the study centre and were, thus, scheduled for monthly drug refill. During the 10-month study period, 265 (81%) children presented to the clinic to collect the prescribed antiretroviral drugs. Of those, 31 caregivers or children did not consent for participation in the study and no plasma was available from two children for performance of VL testing. Two individuals were excluded because they were >18 years at enrolment. A total of 230 HIV-infected children <18 years were, therefore, included in the main analysis. Stored plasma samples for drug measurements were available in 174 (76%) children, and these patients were included in the logistic regression model. A flow chart illustrating inclusion and exclusion of patients is presented in Figure 1. Patients with stored plasma samples did not differ from patients without stored samples with regard to age, sex, $CD4^+$ T-cell count, virological failure rate, self-reported adherence, time on HAART and disclosed HIV infection of mother or father.

Demographic data

Median (IQR) age was 8.8 years (6.1–11.4), 23.5% were below 6, 39.6% between 6 and 9, and 37.0% 10 years or older. Most children were male ($n=121$; 52.8%) Children had been on ART for a median of 3.4 (2.3–4.6) years prior to enrolment. Time on ART increased by 28.3 days (95% CI 7.5, 49.2; $P=0.008$) for every year of age. Most commonly prescribed antiretroviral regimens included zidovudine + lamivudine + NVP in 109 (47.4%) patients, followed by stavudine + lamivudine + NVP in 80 (34.8%) patients. Two children were on concomitant treatment for tuberculosis and both did not undergo plasma drug measurements because of a lack of stored samples. Self-reported adherence was <75% in 15 (7%) children, 75–95% in 35 (15%) children, and >95% in 180 (78%) children (Table 1 and Table 2), and no difference between males and females was noted (χ^2 test, $P=0.44$).

Treatment outcomes

At enrolment, 97 (42%) children had a VL below the detection limit of 50 copies/ml. In total, 12 (5%) children had a VL between 50 copies/ml and 200 copies/ml. A total of 121 (53%) children had a VL>200 copies/ml and met the definition of virological failure. A total of 114 (50%) children had a VL>400 copies/ml. Virological failure was more common in adolescents above the age of 10 years ($n=53$; 63.4%) compared with younger children ($n=68$; 46.9%; $P=0.02$). Overall, median $CD4^+$ T-cell count was 800 cells/mm^3

Figure 1. Flow diagram for patient inclusion/exclusion

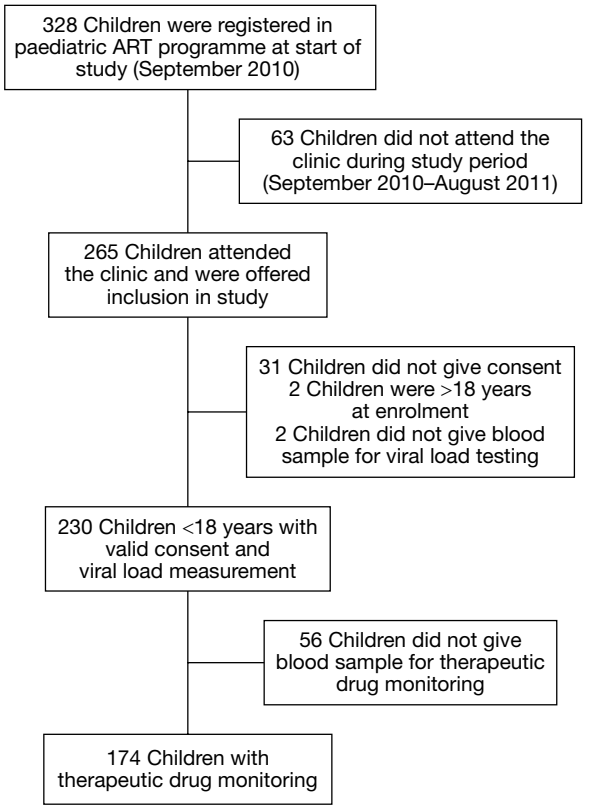


Table 1. Characteristics of patients <18 years enrolled in HIV care at the Bamenda Regional Hospital

Characteristic	No VF (viral load <200 copies/ml)[a]		VF (viral load ≥200 copies/ml)[b]		*P*-value
	Median	IQR	Median	IQR	
Age, years	8.2	5.9–10.6	9.4	6.4–12.3	0.04
Weight, kg	24	19–30	26	20–33	0.10
Height, cm	120	110–130	124	109–137	0.29
$CD4^+$ T-cell count, cells/µl	960	705–1,243	571	261.5–936	<0.001
Aspartate aminotransferase, U/l	38	29–47	39	30–48	0.95
Alanine transaminase, U/l	30	21–37.5	30	22–42	0.22
Age of mother, years	33.5	30–39	34.5	31–40	0.42
Time on ART, years	3.2	2.0–4.3	3.6	2.5–4.9	0.02
NVP concentration, mg/l	8.66	5.13–11.42	6.45	2.92–961	0.02

[a]*n*=109. [b]*n*=121. ART, antiretroviral therapy; NVP, nevirapine; VF, virological failure.

Table 2. Treatment-related characteristics of patients <18 years enrolled in HIV care

Characteristic	No VF (viral load <200 copies/ml)[a]		VF (viral load ≥200 copies/ml)[b]		*P*-value
	n	%	*n*	%	
Age					
0–5 years	29	26.6	25	20.7	0.08
6–9 years	48	44.0	43	35.5	
10–18 years	32	29.4	53	43.8	
Male sex	54	49.5	68	56.2	0.31
WHO stage 3 or 4	3	2.8	10	8.3	0.07
HIV-1/2 coinfection	5	45.5	6	54.6	0.55
Hospitalization in the past 3 months	1	0.9	12	9.9	0.003
Rash	20	18.4	36	29.8	0.04
Vital status of the mother					
Alive	76	69.7	61	50.4	0.01
Dead	33	30.3	59	48.8	
Unknown	0	0.0	1	0.8	
Vital status of the father					
Alive	65	59.6	62	51.2	0.35
Dead	38	34.9	48	39.7	
Unknown	6	5.5	11	9.1	
Previous PMTCT (with sd NVP)					
Done	7	58.3	5	41.7	0.05
Not done	89	50.6	87	49.4	
Unknown	13	31.0	29	69.1	
Adherence level					
<75%	5	4.6	10	8.3	0.49
75–95%	18	16.5	17	14.1	
>95%	86	78.9	94	77.7	
NRTI backbone					
AZT+3TC	63	57.8	72	59.5	0.46
d4T+3TC	42	38.5	42	34.7	
TDF+3TC	3	2.8	7	5.8	
TDF+ddI	1	0.9	0	0.0	
Third drug					
NVP	13	11.9	12	9.9	0.65
EFV	9	8.3	7	5.8	
LPV/r	87	79.8	102	84.3	
Therapeutic drug monitoring					
Subtherapeutic	8	10.5	26	26.5	0.01
Supratherapeutic	36	47.4	30	30.6	
Therapeutic	32	42.1	42	42.9	

[a]*n*=109. [b]*n*=121. AZT, zidovudine; ddI, didanosine; d4T, stavudine; EFV, efavirenz; LPV/r, ritonavir-boosted lopinavir; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PMTCT, prevention of mother to childhood transmission; sd, single dose; TDF, tenofovir; 3TC, lamivudine.

(453–1,108) and counts were significantly lower in children with virological failure.

More adolescents (54.1%) than children <10 years (31.7%; *P*=0.001) reported that their mother had already died. The proportion of children with virological failure was significantly smaller if ART was administered by the mother (41%) compared with ART administration by father, sibling, uncle/aunt or other members of the family (59%; *P*=0.03).

In children below the age of 10, adherence levels were lower when the mother had already died (<75% adherence: 1% versus 13%; 75–95% adherence: 17% in children with mother who is alive versus 8.7% in children with a mother who is dead, *P*=0.01). No such significant association was found in children ≥10 years. Characteristics of patients with and without virological failure are shown in Table 1.

The positive predictive value (PPV) of CD4+ T-cell counts below 200 cells/ mm^3 for virological failure was 100% (95% CI 87.5, 100). PPV of WHO stage 3 or 4 for virological failure was 76.9% (95% CI 49.7, 91.8). The negative predictive values of CD4+ T-cell counts ≥200 cells/mm^3, WHO stage 1 or 2 and good adherence (>95%) for virological success were 53.5% (95% CI 46.3, 60.6), 48.8%(95% CI 42.0, 55.7) and 47.8% (95% CI 40.3, 55.3), respectively.

Logistic regression analysing determinants of virological failure and determinants of poor adherence

In the univariate analysis for the determination of factors for virological failure lower CD4+ T-cell counts, a deceased mother and subtherapeutic plasma levels were significantly associated with virological failure. In the multivariable analysis, male children had 2.2× higher odds to experience virological failure than their female counterparts. Every year on ART conferred a 50% increased risk. An interaction between age and CD4+ T-cell count indicated a more pronounced risk reduction per increase in CD4+ T-cell count in older children. Children who had subtherapeutic drug levels had an almost 4× higher risk to experience virological failure. The positive association between maternal death and failure was similar in strength (Table 3).

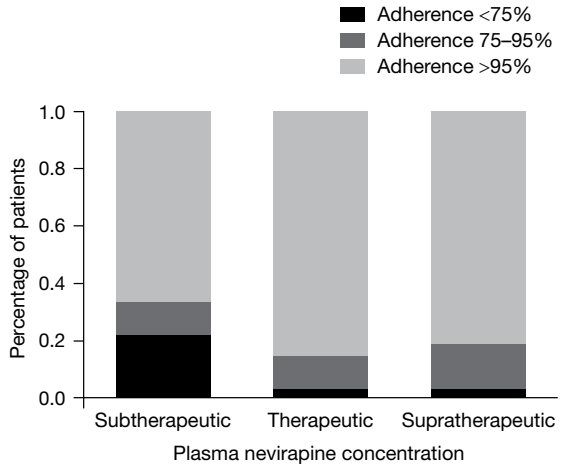
In children >10 years, we found a tendency for higher point estimates of maternal death (OR 15.54; 95% CI 2.46, 98.29), time on ART (OR 2.51; 95% CI 1.16, 5.42) and male sex (OR 5.10; 95% CI 0.75, 34.4), compared with younger children (maternal death OR 1.80;

Table 3. Multivariable logistic regression including selected determinants of virological failure in patients <18 years with available plasma samples for measurement of plasma drug levels

Factor	OR	95% CI	P-value
Univariate analysis			
Male sex	1.59	0.87, 2.92	0.13
Age 0–5 years (versus 10–18 years)	0.48	0.22, 1.05	0.07
Age 6–9 years (versus 10–18 years)	0.59	0.29, 1.20	0.15
CD4+ T-cell count (per 100 cells)	0.82	0.76, 0.89	0.00
Time on ART (per year)	1.17	0.96, 1.41	0.11
Age of mother (per year)	1.02	0.94, 1.1	0.60
Mother dead (versus alive)	2.45	1.30, 4.63	0.01
Father dead (versus alive)	1.40	0.74, 2.67	0.30
HIV-1/2 coinfection (versus HIV-1 monoinfection)	1.42	0.37, 5.44	0.60
Previous PMTCT with sd nevirapine (versus no PMTCT)	0.71	0.18, 2.76	0.62
Hospitalization in previous 3 months	5.77	0.69, 47.94	0.11
WHO stage 3 or 4 (versus stage 1 or 2)	1.59	0.38, 6.56	0.52
NRTI backbone d4T+3TC	0.85	0.46, 1.56	0.59
Adherence <75% (versus >95%)	7.58	0.94, 61.43	0.06
Adherence 75–95% (versus >95%)	1.00	0.42, 2.37	0.99
Subtherapeutic drug levels[a]	3.07	1.30, 7.25	0.01
Multivariable analysis			
Male sex	2.20	1.03, 4.70	0.04
Age 0–5 years (per 100 CD4+ T-cells)	0.86	0.79, 0.94	0.001
Age 6–9 years (per 100 CD4+ T-cells)	0.72	0.64, 0.82	<0.001
Age 10–18 years (per 100 CD4+ T-cells)	0.71	0.61, 0.83	<0.001
Time on ART (per year)	1.50	1.14, 1.96	0.004
Mother dead (versus alive)	4.08	1.74, 9.56	0.001
Subtherapeutic drug levels[a]	3.90	1.25, 12.17	0.02

[a]Subtherapeutic nevirapine, efavirenz or ritonavir-boosted lopinavir plasma levels versus levels above minimum effective concentration. ART, antiretroviral therapy; d4T, stavudine; NRTI, nucleoside reverse transcriptase inhibitor; PMTCT, prevention of mother to childhood transmission; sd, single dose; 3TC, lamivudine.

Figure 2. Self-reported adherence stratified by plasma nevirapine concentrations



P=0.015 (χ^2 test).

95% CI 0.70, 4.65; male sex OR 2.38; 95% CI 0.98, 5.75; time on ART OR 1.10; 95% CI 0.85, 1.42). However, these differences between both age groups did not reach statistical significance (*P* for interaction =0.15, 0.12 and 0.62, respectively). In a secondary analysis for the determinants of poor adherence, WHO stage 3 or 4 was positively associated with low (<75%) adherence (OR 4.13; 95% CI 1.27, 13.45; *P*=0.02). There was a trend for a negative association of higher CD4+ T-cell counts and low adherence (OR 0.94; 95% CI 0.87, 1.00; *P*=0.06). In the multivariable analysis, male sex (OR 1.55; 95% CI 0.80, 3.00; *P*=0.24) and maternal death (OR 0.83, 95% CI 0.43, 1.63; *P*=0.60) were not significantly associated with poor adherence, and this did not vary significantly across age groups (*P* for interaction =0.82 and 0.31, respectively).

A good agreement between low adherence and subtherapeutic plasma levels was found (kappa interrater agreement: 74%; *P*=0.02).

NVP levels

A total of 189 (82%) children received NVP as part of their ART. Stored plasma samples for the analysis of NVP plasma levels were available in 154 (81.5%) of these patients. In total, two children had been prescribed an incorrect dose of NVP and were excluded from further analyses of NVP plasma levels. Out of 152 children, 27 (17.8%) patients receiving NVP had subtherapeutic plasma levels below 3 mg/l (group A), 63 (41.5%) had supratherapeutic levels (group B) and 62 (40.8%) had plasma levels in the therapeutic range (group C).

Group C (normal range) and B (supratherapeutic) were associated with better adherence than group A (subtherapeutic): 85.5%, 81% and 66.7% had >95% adherence, respectively. Children in group A had a higher VL (median 4.3 log copies/ml) than the other groups (2.9 and 3.2 log copies/ml in group B and C, respectively; *P*=0.001). There was a trend towards lower CD4+ T-cell counts in group A compared with group B or C (median 547 cells/mm^3 versus 824 and 838 cells/mm^3, respectively; *P*=0.09).

No significant between-group differences were noted in terms of age, sex, weight and height, liver function tests and the presence of rash or other side effects. In contrast to the outcome virological failure, no difference regarding maternal death was noted between the three groups, even when stratified by age above or below 10 years. Gender was not associated with NVP plasma concentrations (Student's *t*-test, *P*=0.33).

The proportion of self-reported adherence according to subtherapeutic, supratherapeutic and therapeutic NVP plasma concentrations is depicted in Figure 2.

A total of 47 (31%) children had a NVP plasma concentration ≥10 mg/l, 4 children had ≥20 mg/l, and 1 of these children even had a plasma concentration of 67 mg/l. Liver function tests were increased in three patients (grade 1–2), and three patients reported papular skin rash (grade 2; Table 4). There was no significant increase in side effects, including rash, diarrhoea and fever, or increase of liver function tests reported in this group compared with children with normal or subtherapeutic levels (*P*=0.45).

Discussion

In this cross-sectional study, we found that more than half (53%) of children in a routine ART programme in rural Cameroon experienced virological failure (>200 copies/ml) after having received ART for a median of 3.4 years. To our knowledge, this is the first report to demonstrate such high failure rates in children in Central Africa. In addition, 12 (5.3%) children had a VL greater than 50 copies/ml but lower than 200 copies/ml, which could represent viral blips without clinical relevance but may also include some virological failures detected early in this cross-sectional setting [28]. The rate of virological failure found in our study is substantially higher than that reported from other parts of Africa [29,30] For example, in South Africa, where virological monitoring is routinely performed, virological failure rates among children 3 years after initiating ART were 19%. This suggests a need to identify country-specific obstacles and define strategies to improve treatment outcomes.

Factors significantly associated with virological failure included time on ART, CD4+ T-cell counts, sex, maternal death and antiretroviral drug levels of NVP, EFV and LPV/r. Longer time of ART use was shown

Table 4. Children with excessively high[a] NVP plasma concentrations[b]

ID	Age, years	Sex	Status of mother	Weight, kg	Height, cm	HAART	NVP[c], mg	NVP[d], mg/l	Medication[e]	Symptoms[f]	CD4[g]	Viral load, copies/ml	ASAT, U/l	ALAT, U/l
1	5.9	M	Dead	21	113	Triomune[h] 1–0–0.5	300	23.4	No	Fever, cough, vomiting, diarrhoea	1,077	21,085	103	434
2	10.4	F	Dead	38	146	Zidovex[i] 1–0–1	400	24.0	No	None	831	1,391	33	28
3	9.5	F	Alive	30	127	Zidovex[i] 1–0–1	400	34.2	No	Lymphadenopathy, discharging ear	922	<50	40	27
4	11.6	M	Alive	25	121	Triomune[h] 1–0–1	400	67.3	No	Cough, fever, papular skin rash	432	<50	33	19

[a]≥20 mg/l. [b]All children received correct doses according to WHO recommendations [20]. [c]Daily dose. [d]Plasma concentration. [e]Concomittant. [f]Reported clinical. [g]CD4+ T-cell count cells/mm^3. [h]Fixed-dose drug combination of stavudine 30 mg, lamivudine 150 mg and nevirapine (NVP) 200 mg. [i]Fixed-dose drug combination of zidovudine 300 mg, lamivudine 150 mg and NVP 200 mg. ALAT, alanine transaminase; ASAT, aspartate aminotransferase.

to be associated with higher rates of virological failure in previous studies [10]. In our study, age and time on ART were positively correlated, and the trend for a higher risk for virological failure in older children may be explained by accumulating drug resistance in light of insufficient monitoring of treatment [31]. The association of higher CD4+ T-cell counts and less risk for virological failure found in our study was less pronounced in children <5 years, most probably because of the higher number of absolute CD4+ T-cells in this infant population. Lower CD4+ T-cell counts are commonly observed in the presence of detectable VL and HIV progression [32] and are rather a consequence than a cause of virological failure. The finding that male sex is associated with more than a double risk for virological failure in children is previously unreported. Male sex was previously associated with poorer treatment outcomes in adults from South Africa [33] and Malawi [34] and was thought to be as a result of poorer adherence. In our study, we could not find an association of gender and self-reported adherence; however, adherence over a longer period was not assessed.

In the univariate analysis, self-reported adherence and maternal death seemed to be associated in younger children in whom the intimate interaction between mother and child may be particularly important for treatment success.

Risk for virological failure was substantially increased if the mother had already died. In a sensitivity analysis stratified by age, we found that the association of maternal death and virological failure seemed to be stronger in older children (≥10 years), although this difference across both age groups was not significant and warrants further verification in larger studies. Adolescence may be a vulnerable period, and factors, such as higher levels of malnutrition and later initiation of ART with more advanced immunodeficiency, may occur in the absence of a mother as a primary carer and may contribute to treatment failure [10]. Longer duration of infection and ART use in mother and child with the risk of development of drug resistance, virological failure and death [35] may be an alternative explanation.

The stigmatization of disclosing the child's HIV status to other members of the family who might be HIV uninfected could equally lead to adverse outcomes [36]. Consequently, children who received ART from a family member other than the mother had higher virological failure rates. Subtherapeutic plasma levels of the antiretroviral drugs NVP, EFV and LPV/r were also independently associated with failure, which is in-line with previous reports [25,26] and indicates that efforts to strengthen adherence should result in higher virological success rates. Poor adherence was associated with WHO stage 3 or 4 in our study, which could indicate less motivation for adherence in sicker children. However, progressive disease could also be a consequence of incomplete adherence, which we cannot distinguish because of the cross-sectional design of our study.

Similar to previous reports [37,38], we found a high variability of NVP levels, which was by far the most commonly prescribed non-nucleoside reverse transcriptase inhibitor in our study. Potential explanations include unreported traditional co-medications or irregularities in drug intake and possibly exceeding intake before presentation to the clinic. In total, 18% of children receiving NVP had subtherapeutic plasma levels, and these children were significantly more likely to be virologically failing on treatment. We observed supratherapeutic NVP levels in a substantial proportion of children (42%). This might be explained by a decreased NVP clearance [39] or polymorphisms of CYP*2B6* and *3A4* genes, which have been shown to influence NVP plasma concentration in African children [40–42]. High NVP levels have also been associated with a higher rate for hepatotoxicity [43] and rash [44], although we did not observe an increased risk for adverse events in

children with supratherapeutic drug levels in our setting. At the time of the study, all children on NVP were receiving the drug for at least 6 months, a time after which cutaneous hypersensitivity reactions are uncommon [39]. However, ongoing vigilance for hepatotoxicity, which may be dose-dependent and increases with time, seems to be important [45].

Strengths of the study include the large sample size and the fact that this dataset provides information of a real-life scenario under programmatic conditions, including a high coverage of children still in care after several years. We think that this situation may be representative for other ART programmes in Cameroon and Central Africa. The study had several limitations: firstly, adherence was assessed by self-report only, and pill counting or medication event monitoring were not performed. Drug adherence in the previous 28 days as assessed in our study may not represent adherence over longer periods. Previous pharmacy refill records, which have been shown to measure adherence well [46], were found to be incompletely available in medical files and were, thus, not included in this cross sectional analysis. This could have resulted in bias when patients reported a better than real adherence because they found this more desirable. In contrast to previous reports [14,47], we found a good correlation between self-reported adherence and plasma drug levels, indicating that self-reported adherence may be an appropriate indicator in this setting. However, only approximately half of children who reported good adherence had, in fact, virological success. Secondly, the exact time of drug intake was not accurately recorded. Hence we could not precisely calculate when plasma levels were taken after last drug intake, which could have led to an underestimation of the subtherapeutic plasma levels of LPV/r and EFV. However, exact timing of drug intake does not seem to play a role for the interpretation of NVP plasma concentrations because differences between peak and trough concentrations are small once a steady state has developed [48]. We used a cutoff of 3 mg/l, which has been shown to predict virological failure in a random selection of patients with unknown time of NVP intake [25]. Lastly, drug resistance, which is a well-known risk factor for virological failure was not yet assessed in this study. It was previously shown that 90% of children failing first-line ART in Cameroon have major drug mutations partly related to the widespread use of single-dose NVP to prevent mother-to-child transmission [17] and that treatment outcomes are inferior in children receiving NVP compared with LPV/r [49]. In our study, 74% of children with virological failure had therapeutic or supratherapeutic drug levels, and it is possible that drug resistance already developed in a substantial number of these children. In addition, particular HIV-1 subtypes which allow rapid selection of resistance [50] to antiretroviral drugs used in first-line regimens could have contributed to the virological failure rates found in our study.

In summary, we report high virological failure rates among HIV-infected children under programmatic conditions in rural Cameroon and identified maternal death, male sex and longer time on ART as important determinants of failure. Subtherapeutic drug levels were associated with higher risk for virological failure, indicating a strong need to promote adherence to achieve better outcomes in this special patient population. In children receiving NVP, the variability of plasma levels indicates the possibility of an irregular drug intake or a role of genetic factors, and this finding warrants further investigation. Intensified adherence counselling and stringent monitoring of accurate drug intake seem necessary to improve success rates in this resource-limited setting.

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