Predictors of Treatment Failure in HIV-Positive Children Receiving Combination Antiretroviral Therapy: Cohort Data From Mozambique and Uganda

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Background. Delays detecting treatment failure and switching to second-line combination antiretroviral therapy (cART) are often observed in human immunodeficiency virus (HIV)–infected children of low-middle-income countries (LMIC).

Methods. An observational study included HIV-infected children attending the Beira Central Hospital (Mozambique) and the Nsambya Hospital, Home Care Department (Uganda) evaluated clinical and immunological failure according to World Health Organization (WHO) 2006 guidelines. Baseline predictors for cART failure and for drug substitution were explored in unadjusted and adjusted Cox proportional hazard models.

Results. Two hundred eighteen of 740 children with at least 24 weeks follow-up experienced treatment failure (29%; 95% confidence interval [CI] 26–33), with crude incidence of 20.0 events per 100 person-years (95% CI 17.5–22.9). Having tuberculosis co-infection or WHO stage 4, or starting a nontriple cART significantly increased risk of failure. Two hundred two of 769 (26.3%) children receiving cART substituted drug(s), with crude incidence of 15.4 events per 100 person-years (95% CI 13.4–17.7). Drug toxicity (18.3%), drug availability (17.3%), and tuberculosis drugs interaction (52, 25.7%) were main reported reasons, while only 9 (4%) patients switched cART for clinical or immunological failure. Children starting lamivudine-zidovudine-nevirapine or lamivudine-stavudine-efavirenz or lamivudine-zidovudine-efavirenz were more likely to have substitute drugs. Increased substitution was found in children with mild immunosuppression and tuberculosis co-infection at cART initiation as well as poor adherence before drug substitution.

Conclusions. Considerable delay in switching to second-line cART may occur despite an observed high rate of failure. Factors including WHO clinical stage and tuberculosis co-infection should be evaluated before starting cART. Toxicity and drug adherence should be monitored to minimize drug substitution in LMIC.

Key words. children; drug substitution; HIV; treatment failure.

BACKGROUND

The global scaling up of treatment and care for people living with human immunodeficiency virus (PLWH) has led to a 43% decline in new HIV pediatric infections since 2003, with 330,000 newly infected children in 2011. Despite efforts to expand access to combination antiretroviral therapy (cART), only 28% of eligible children have received it [1]. Expansion of early HIV diagnosis coverage, prompt cART initiation, and better retention in care remain major goals [2, 3], and the lack of laboratory monitoring frequently observed in low and middle-income countries (LMIC) should not represent a barrier to cART distribution in children [4]. However, optimization of the clinical management of PLWH and prompt diagnosis of treatment failure are becoming increasingly critical in the context of lifelong treatment and limited drug availability.

Although virological failure is widely considered the criterion standard to detect treatment failure, clinical and immunologic parameters are often the only criteria available in LMIC [5, 6]. CD4 cell monitoring has been shown to be a poor predictor of virological failure in treatment-experienced children [7–9], particularly when severely immune-compromised [10]. Studies in LMIC have reported high rates of virological suppression in children up to 5–6 years after treatment initiation [11, 12]; however, treatment failure rates of 10–34% were observed among children after 2–3 years of cART [13–18]. Program reports suggest that only a small proportion of patients on treatment are receiving a second–line therapy, an estimated 4% of adults and 1–14% of children [16, 18–20]. Delays in detecting treatment failure and switching to second-line therapy lead to the development of HIV drug-resistance, compromising subsequent regimens [6, 21]. This is particularly relevant for children, due to the lack of pediatric formulations.

Randomized trials were conducted to evaluate the optimal first antiretroviral regimen for reducing the risk of treatment failure. Findings from the P1060 trial reported an increased risk of failure starting a nevirapine (NVP)-based cART in infants and young children [13, 22, 23]. This was not confirmed by the PENPACT1 trial, where no difference in clinical and virological outcomes were shown between non-nucleoside reverse-transcriptase inhibitors (NNRTI) and protease inhibitors (PI)–based regimens in older children [24]. Data to inform the most durable nucleoside reverse-transcriptase inhibitors (NRTI) backbone in the context of a triple therapy is still limited. Conflicting results were reported concern the use of abacavir (ABC) as first-line regimen: Green et al. suggested that abacavir (ABC) may be preferable to zidovudine (AZT) combination with lamivudine (3TC) [25], while poorer early virological outcomes were recently observed in children starting ABC/3TC-based first-line regimens, compared to stavudine (d4T)/3TC [26, 27]. Identifying optimal regimens is particularly relevant for children with HIV/tuberculosis (TB) co-infection living in LMIC, where NVP is widely preferred to efavirenz (EFV) or a triple NRTI-based regimen, due to its better acceptability and relatively low cost [28].

Drug substitution is often required to optimize antiretroviral treatment [19, 29]. Results from observational studies estimate a probability of cART discontinuation or modification ranging between 2.8% and 20% in adults of LMIC [19, 30–34]. A randomized study conducted in children shows a cART switching/discontinuation rate up to 29% [24]. Acute and chronic toxicity, drug intolerance, poor adherence, and treatment failure remain the major determinants of cART modification [35–38]. Drug costs and/or being out of stock due to challenges in adequately forecasting and maintaining an effective supply chain have been cited as further reasons for cART discontinuation in LMIC [31, 33].

The aim of this study is to estimate the rate and predictors of cART treatment failure in 2 pediatric cohorts from Mozambique and Uganda during a 5-year follow-up period, and to explore the rate of and factors associated with drug substitution.

METHODS

Setting and Study Design

We conducted a retrospective cohort study among children starting cART between January 2005 and December 2009 at the Beira Central Hospital (HCB) in Mozambique and the Nsambya Home Care (NHC) department of St. Raphael of St. Francis Hospital in Uganda. Two Italian nongovernmental organizations, Doctors with Africa Cuamm (Mozambique) and Associazione Casa Accoglienza alla Vita Padre Angelo (Uganda), partnered with these hospitals to provide pediatric HIV care.

Both programs provided HIV counseling and testing, cotrimoxazole prophylaxis, cART, laboratory investigations, and management of opportunistic infections. Infants and children under 18 months of age, known or suspected to be exposed to HIV, were diagnosed through HIV-1 DNA testing. Patients were considered eligible for cART according to WHO 2006 guidelines [39].

Laboratory examinations including full blood count, liver function tests, creatinine, and CD4 count were required before starting cART, as well as a chest radiograph and acid-fast bacilli testing to exclude TB if suspected. In the absence of contraindications, written consent was collected when enrolling in the programme and before starting cART. Throughout the study, patients were switched to second-line cART when treatment failure was identified following WHO 2006 guidelines [39].

The study was approved by the ethics committees of HCB and Nsambya Hospital and registered by the Uganda National Council for Science and Technology and by the Gabinete Do Director Gerar, Ministerio Da Saudè of HCB (Mozambique).

Data Collection. In Mozambique, data were collected from clinical charts and paper registries and entered in the hospital's electronic patient database system. Similarly, in Uganda, routine clinical data were recorded in paper-based patient files and registries and entered into an electronic interface by trained staff.

Children were examined at least monthly during the first 6 months of cART and then every 3 months in Mozambique, while in Uganda monthly visits were

maintained throughout the follow-up according to the project design. Weight and height were measured at every clinic visit. Full blood count, liver function tests, and glucose assays were performed every 6 months, and CD4 counts every 6–12 months. Adherence to cART was assessed at every follow-up visit and defined as "good" or "poor" if the self-reported number of doses was more or less than 95% of expected monthly number of doses. HIV-related clinical events were diagnosed with or without biological confirmation, depending on lab facilities available, while immunodeficiency was classified as mild, advanced, and severe according to the WHO 2006 thresholds [39]. For the treatment failure analysis, the period of follow-up was from cART initiation up to the treatment failure outcome, while follow-up was from treatment initiation to first cART drug substitution for drug-substitution analysis. For children without treatment failure or drug substitution, follow-up was censored at date of death, loss to follow-up (LTFU, defined as missing follow-up visits for more than 6 months), transferred to other clinic, confirmed HIV-negative or aged more than 18 years old, last CD4 measurement, or last anthropometric or adherence record, whichever occurred latest.

Endpoint Definitions and Study Population. Drug-substitution was defined as substitution of one or more drugs of the first antiretroviral regimen for any reason. Reasons for drug substitution were classified retrospectively from the inspection of what was reported by clinicians in patient's clinical charts. Clinical and immunological failure were defined according to the WHO 2006 criteria, using CD4 measurements and WHO disease stage from at least 24 weeks after cART initiation [39]. Treatment failure, when both clinical and immunological failures were observed, was considered to occur at the earliest of the two events.

For analysis of treatment failure, only children with at least 24 weeks of follow-up post-cART initiation were included to ensure sufficient time for treatment response.

For analysis of cART drug substitution, children who received an ABC component in their initial cART regimen were excluded, as first-line ABC treatment was systematically administered to children diagnosed with active TB and all patients initially on ABC were routinely switched to EFV once the TB infection cleared.

Statistical Analyses. In this intent-to-treat analysis, all children were included from cART initiation, regardless of subsequent modifications. All analyses were conducted in R version 2 (R Development Core Team, Vienna, Austria) and Stata version 12.0 (Stata Corporation, College Station, TX).

For both treatment failure and drug-substitution analyses, frequency distributions and median and interquartile range (IQR) were used to describe baseline patient characteristics. Baseline characteristics of interest were gender, age at treatment initiation, body–mass index (BMI, weight[kg]/height^2[m]) for age z-score, WHO disease stage, initial cART treatment regimen (also by most potent component), adherence to cART, CD4 count and percent, and immunodeficiency classification. All descriptive analyses were stratified by hospital. Differences in all key variables at baseline between these strata were determined using Pearson's χ^2 test for categorical variables, the t-test for difference in means for baseline BMI for age z-score, and the Wilcoxon rank-sum test for all other continuous variables.

Unadjusted Cox proportional hazards models were used to determine the odds of treatment failure and cART drug substitution. The following variables were considered in a multivariate adjusted Cox proportional hazards model of treatment failure: cART treatment regimen, age, adherence, gender, country of treatment, baseline disease stage, immunodeficiency status, and BMI for age z-score. The following variables were considered in a multivariate adjusted Cox proportional hazards model of cART drug substitution: cART treatment regimen, adherence, classification of immunodeficiency status, WHO disease stage, and age group. A backward-selection procedure was used to create these adjusted models, with a variable being included in the model if it resulted in an improvement in the model fit as defined by the Akaike Information Criterion (AIC).

RESULTS

Between January 2005 and December 2009, 1075 HIV-infected children less than 15 years old began cART in HCB and NHC. Two hundred thirteen (20%) children were excluded from the study due to missing data (Table 1). Children excluded from both treatment-failure and drug-substitution analyses were more likely to be Ugandan ($P < 0.01$), female ($P = 0.049$), younger ($P < 0.01$) and enrolled and starting cART later ($P < 0.01$ and < 0.01, respectively) than children included in the study.

Treatment Failure Analyses

Among 862 children eligible for analysis, 740 children (492 from Mozambique and 248 from Uganda) with at least 24 weeks of follow-up were included for a total of 1088.5 person/years of follow-up. At the time of data collection, 24/740 (3.24%) children died, 68 (9.19%) were LTFU, 7 (0.95%) were transferred to another clinic, and 1 (0.14%) was confirmed HIV-negative. A total of 218 treatment failure events (29%; 95% CI 26–33) occurred, with a crude incidence rate of 20.0 events per 100 person-years (95% CI 17.5–22.9). Median time to treatment

Table 1. Baseline Characteristics of 862 Children Included and 213 Children Excluded from Analyses Due to Missing Data

Variable	Value	Included children n = 862	Excluded children n = 213 (20%)	P value
Gender	Male	449 (52)	99 (46)	
	Female	413(48)	113 (53)	0.049[a]
Country	Mozambique	583 (68)	102 (48)	
	Uganda	279 (32)	111 (52)	<0.01[a]
Year of birth	Median (IQR)	2002 (1998–2005)	2001 (1996–2005)	
	<1995	76 (9)	34 (16)	0.02[b]
	1995-1999	219 (25)	54 (25)	
	2000-2004	289 (34)	58 (27)	<0.01[a]
	2005-2009	278 (32)	59 (28)	
	missing	0 (0)	8 (4)	
Age at treatment initiation (years)	Median (IQR)	4.83 (2.09–9.11)	6.33 (2.62–11.56)	
	<12 months	62 (7)	11 (5)	<0.01[b]
	12–35 months	256 (30)	48 (23)	
	36–59 months	120 (14)	22 (10)	
	5–8 years	157 (18)	40 (19)	<0.01[a]
	>8 years	267 (31)	92 (43)	
	Missing	0 (0)	8 (4)	
WHO clinical stage at treatment initiation	Stage I or II	75 (9)	14 (7)	
	Stage III with TB	36 (4)	11 (5)	
	Stage III w/o TB	57 (7)	22 (10)	
	Stage IV with TB	10 (1)	6 (3)	0.14[a]
	Stage IV w/o TB	47 (6)	13 (6)	
	Unknown	637 (74)	147 (69)	
Age at enrollment (years)	Median (IQR)	3.94 (1.48–8.09)	5.57 (1.61–9.47)	
	Missing	0 (0)	8 (4)	0.01[b]
Initial treatment regimen	3TC + 4DT + NVP	369 (43)	70 (33)	
	3TC + AZT + NVP	231 (27)	60 (28)	
	3TC + AZT + EFV	80 (9)	35 (16)	
	3TC + 4DT + EFV	57 (7)	15 (7)	
	3TC + AZT + ABC	48 (6)	15 (7)	0.06[a]
	Other triple	68 (8)	15 (7)	
	Other dual	3 (0)	1 (<1)	
	missing	6 (1)	2 (1)	

Abbreviations: ABC, abacavir; AZT, zidovudine; 4DT, stavudine; EFV, efavirenz; IQR, interquartile range; NVP, nevirapine; TB, tuberculosis; 3TC, lamivudine; WHO, World Health Organization.

[a]Pearson χ^2 test.

[b]Kruskal-Wallis test.

failure was 379 days (IQR 229–649). Immunological failure alone occurred in 100 (46%) children, while clinical failure alone was found in 116/218 (53%) cases. Two children (1%) had concomitant clinical and immunological failure. Baseline characteristics are shown in Table 2.

The adjusted Cox proportional hazards model of treatment failure with the lowest AIC included age, treatment type, and baseline disease stage. Incidence rates and crude and adjusted relative hazards from the model are shown in Table 3.

Patients with TB and those with other WHO stage 4 defining diseases were significantly more likely to experience treatment failure (Hazard Ratio [HR] 2.27, 95% CI 1.5–3.4, P < 0.01 and HR 1.57, 95% CI 1.02–2.4, P = 0.04, respectively) compared to children with WHO stage 3 disease without TB. As expected, starting cART with an unconventional regimen (not containing an NRTI backbone in combination with EFV, NVP, lopinavir/ritonavir (LPV/r), or ABC) was also significantly associated with risk of treatment failure (HR 3,37, 95% CI 1.12–11.89, P = 0.03).

Drug Substitution Analysis. Among 862 eligible children, 4 with unknown ART regimen and 89 who received ABC in their initial cART regimen were excluded from the cART drug-substitution analysis. The remaining 769 children had an overall follow-up of 1499 person/years. Baseline characteristics of this cohort are provided in Supplementary Table 1 (see online Supplementary Material for this table).

Throughout the study period, 202 (26%, 95% CI 23–30) patients substituted treatment, with median time to substitution of 9.69 months (IQR 25.82). Overall incidence of substitution was 15.4 events per 100 person-years (95% CI 13.4–17.7). Reported reasons for substitution included any toxicity (37, 18.3%), of which 3 were d4 T toxicity (1.5%), 25 (12.4%) AZT toxicity, and 9 (4.5%) NVP toxicity, clinical and immunological failure (9, 4.5%), drug availability (35, 17.3%), drug interaction (1, 0.5%), provider preference for a better option (32, 15.8%), simplification associated with nonadherence (4, 2%), caregiver health problem (1, 0.5%), and TB drugs interaction (52, 25.7%). Among the 9 patients with drug substitution for clinical or immunological failure, median time to

Table 2. Baseline Characteristics of Children Included in the Treatment Failure Analysis: Demographics and Treatment

Variable	Value	All children n = 740 (100.00%)	Mozambique n = 492 (66.49%)	Uganda n = 248 (33.51%)	P value[a]
Gender	Male	382 (51.62)	260 (52.85)	122 (49.19)	0.348
	Female	358 (48.38)	232 (47.15)	126 (50.81)	
Age at treatment initiation (n = 205)	Median (IQR)	5.05 (7.00)	3.42 (5.49)	8.22 (7.13)	<0.01
	<12 months	51 (6.89)	44 (8.94)	7 (2.82)	<0.01
	12-35 months	216 (29.19)	186 (37.80)	30 (12.10)	
	36-59 months	97 (13.11)	68 (13.82)	29 (11.69)	
	>5 years	376 (50.81)	194 (39.43)	182 (73.39)	
BMI for age z-score		−0.92 (1.94)	−1.10 (1.95)	−0.75 (1.82)	
	Median (IQR)	(n = 564)	(n = 327)	(n = 237)	0.019
WHO disease stage	Stage I or II	174 (23.51)	46 (9.35)	128 (51.61)	<0.01
	Stage III with TB	83 (11.22)	68 (13.82)	15 (6.05)	
	Stage III w/o TB	305 (41.22)	224 (45.53)	81 (32.66)	
	Stage IV with TB	52 (7.03)	48 (9.76)	4 (1.61)	
	Stage IV w/o TB	101 (13.65)	81 (16.46)	20 (8.06)	
	Unknown	25 (3.38)	25 (5.08)	0 (0.00)	
Initial treatment regimen	3TC + d4T + NVP	325 (43.92)	269 (54.67)	56 (22.58)	<0.01
	3TC + AZT + NVP	195 (26.35)	114 (23.17)	81 (32.66)	
	3TC + AZT + EFV	69 (9.32)	13 (2.64)	56 (22.58)	
	3TC + d4T + EFV	50 (6.76)	24 (4.88)	26 (10.48)	
	3TC + AZT + LPV/r	18 (2.43)	0 (0.00)	18 (7.26)	
	3TC + d4T + LPV/r	6 (0.81)	0 (0.00)	6 (2.42)	
	3TC + d4T + ABC	25 (3.38)	25 (5.08)	0 (0.00)	
	3TC + AZT + ABC	40 (5.41)	39 (7.93)	1 (0.40)	
	Other[b]	12 (1.62)	8 (1.63)	4 (1.61)	
Initial treatment regimen (by most potent component)	EFV-containing	120 (16.22)	37 (7.52)	83 (33.47)	<0.01
	NVP-containing	523 (70.68)	383 (77.85)	140 (56.45)	
	LPV/r-containing	24 (3.24)	0 (0.00)	24 (9.68)	
	ABC-containing[c]	67 (9.05)	66 (13.41)	1 (0.40)	
	Other[d]	6 (0.81)	6 (1.22)	0 (0.00)	
Adherence	Good	485 (65.54)	294 (59.76)	191 (77.02)	<0.01
	Poor	255 (34.46)	198 (40.24)	57 (22.98)	
CD4 percent (mean, 95% CI)	<12 months	15.95 (9.60)	15.60 (9.20)	18.93 (4.60)	<0.01
	12-35 months	14.50 (8.70)	15.00 (8.20)	10.62 (8.12)	
	36-59 months	12.16 (7.90)	12.85 (7.05)	11.81 (8.77)	
	>5 years	9.60 (10.00)	11.05 (10.00)	8.47 (9.76)	
CD4 count (mean cells/mm³, 95% CI)	<12 months	784.00 (971.00)	746.50 (765.50)	1404.00 (1145.00)	<0.01
	12-35 months	721.00 (606.00)	730.50 (585.00)	554.00 (765.00)	
	36-59 months	467.00 (395.50)	420.50 (292.50)	551.00 (509.00)	
	>5 years	239.00 (286.00)	265.00 (363.00)	226.00 (224.00)	
CD4 count z-score	Median (IQR)	−0.30 (1.07)	−0.14 (1.12)	−0.54 (0.69)	<0.01
		(n = 736)	(n = 492)	(n = 244)	
Classification of immunodeficiency[e]	Not significant	58 (7.84)	40 (8.13)	18 (7.26)	0.092
	Mild	35 (4.73)	19 (3.86)	16 (6.45)	
	Advanced	66 (8.92)	37 (7.52)	29 (11.69)	
	Severe	581 (78.51)	396 (80.49)	185 (74.60)	

Abbreviations: ABC, abacavir; AZT, zidovudine; BMI, body–mass index; CI, confidence interval; d4 T; EFV; IQR, interquartile range; LPV/r; NVP, nevirapine; TB, tuberculosis; 3TC, lamivudine; WHO, World Health Organization.

[a]P values refer to differences between Mozambique and Uganda subcohorts on baseline characteristics.

[b]Other regimens include mono or dual therapies and those with missing information on combination antiretroviral therapy regimen.

[c]ABC-containing regimen includes a 3 NRTI regimen containing ABC.

[d]Other regimens include only those without an EFV, NVP, LPV/r, or ABC component, regardless of number of components.

[e]Immunodeficiency was classified as mild (CD4% of 30–35, 25–30, 20–25 and CD4 cell count of 350–499 for children ≤11 months, 12–35 months, 36–59 months or ≥5 years, respectively), advanced (CD4% of 25–29, 20–24, 15–19 and CD4 cell count of 200–349 for children ≤11 months, 12–35 months, 36–59 months or ≥5 years, respectively) and severe (CD4% <25, <20, <15 and CD4 cell count <200/<15% for children ≤11 months, 12–35 months, 36–59 months or ≥5 years, respectively) according to the WHO 2006 thresholds.

substitution was 26.65 months (IQR 23.95). Reasons for substitution were unknown for 31 (15.3%) children.

Drug substitution was more likely among patients starting 3TC-AZT-NVP (adjusted HR 3.29, 95% CI 2.27–4.76, P < 0.01), 3TC-d4T-EFV (adjusted HR 3.22, 95% CI 2.02-5.13, P < 0.01), or 3TC + AZT + EFV (adjusted HR 1.74, 95% CI 1.03–2.95, P = 0.037) compared to 3TC-d4T-NVP.

Mildly immunosuppressed patients (adjusted HR 2.23, 95% CI 1.24–4.02, P < 0.01), infants (adjusted HR 2.74, 95% CI 1.54–4.90, P < 0.01), children with TB (adjusted HR 3.38, 95% CI 2.28–5.01, P < 0.01) and those with good treatment adherence before drug substitution (adjusted HR 0.53, 95% CI 0.37–0.77, P < 0.01) were also more likely to substitute cART. Incidence rates and crude and adjusted

Table 3. Relative Hazards for Treatment Failure in Children From Mozambique and Uganda ($n = 740$ Children)

Variable		Person time (years)	Events	Crude incidence rate[a] (95% CI)	Unadjusted relative hazard	95% CI	P value	Adjusted relative hazard	95% CI	P value
Treatment type	NVP-containing	781.2	150	19.2 (16.4,22.5)	Reference			Reference		
	ABC-containing[b]	62.0	18	29.0 (18.3,46.1)	1.38	(0.84,2.25)	0.20	0.76	(0.43,1.34)	0.34
	EFV-containing	187.5	37	19.7 (14.3,27.2)	1.09	(0.76,1.56)	0.65	0.95	(0.64,1.41)	0.80
	LPV/r-containing	53.8	10	18.6 (10.0,34.5)	1.08	(0.56,2.08)	0.81	1.03	(0.53,2.02)	0.93
	Other[c]	3.9	3	76.1 (24.6,236.1)	3.32	(1.05,10.43)	0.04	3.73	(1.17,11.89)	0.03
BMI for age z-score tertiles	Lowest tertile	410.7	96	23.4 (19.1,28.6)	Reference					
	Middle tertile	260.6	42	16.1 (11.9,21.8)	0.69	(0.48,0.99)	0.04			
	Highest tertile	154.1	38	24. 7 (17.9,33.9)	1.04	(0.71,1.51)	0.85			
	Unknown	263.1	42	16.0 (11.8,21.6)	0.68	(0.48,0.98)	0.04			
Gender	Female	540.7	100	18.5 (15.2,22.5)	Reference					
	Male	547.8	118	21.5 (18.0,25.8)	1.17	(0.89,1.52)	0.26			
Country of treatment	Mozambique	681.5	143	21.0 (17.8,24.7)	Reference					
	Uganda	407.0	75	18.4 (14.7,23.1)	0.91	(0.69,1.21)	0.51			
Adherence	Good	1027.3	202	19.7 (17.1, 22.6)	Reference					
	Poor	61.1	16	26.2 (16.0, 42.7)	1.24	(0.74,2.06)	0.41			
Classification of immunodeficiency[d]	Not significant	78.6	10	12.7 (6.8,23.6)	0.58	(0.31,1.10)	0.10			
	Mild	57.9	11	19.0 (10.5,34.3)	0.89	(0.49,1.64)	0.72			
	Advanced	90.3	13	14.4 (8.4,24.8)	0.64	(0.36,1.12)	0.12			
	Severe	861.7	184	21.4 (18.5,24. 7)	Reference					
Age group	0–11 months	46.0	13	28.2 (16.4,48.6)	1.28	(0.72,2.23)	0.41	1.08	(0.59,1.96)	0.80
	12–35 months	275.5	64	23.2 (18.2,29.7)	1.12	(0.83,1.52)	0.46	1.08	(0.77,1.50)	0.66
	35–59 months	176.8	23	13.0 (8.6,19.6)	0.67	(0.43,1.05)	0.08	0.64	(0.41,1.00)	0.05
	≥5 years	590. 1	118	20.0 (16.7,24.0)	Reference			Reference		
WHO disease stage at baseline	Stage 1 or 2	289.0	52	18.0 (13.7,23.6)	1.12	(0.79,1.58)	0.52	1.17	(0.81,1.67)	0.40
	Stage 3 or 4 with TB	145.8	50	34.3 (26.0,45.2)	1.96	(1.38,2.79)	<0.01	2.27	(1.50,3.42)	<0.01
	Stage 3 w/o TB	510.9	83	16.2 (13.1,20.1)	Reference	(0.78,7.7)	0.045	Reference		
	Stage 4 w/o TB	106.3	29	27.3 (19.0,39.3)	1.54	(1.01,2.35)	0.40	1.57	(1.02,2.414)	0.04
	Unknown	36.5	4	11.0 (4.1,29.2)	0.65	(0.24,1.78)		0.67	(0.24,1.82)	0.43

Abbreviations: ABC, abacavir; BMI, body–mass index; CI, confidence interval; EFV, efavirenz; LPV/r, lopinavir/ritonavir; NVP, nevirapine; WHO, World Health Organization.

[a]Per 100 years.

[b]ABC-containing regimen include a 3 NRTI regimen containing ABC.

[c]Other regimens include only those without an EFV, NVP, LPV/r, or ABC component, regardless of number of components.

[d]Immunodeficiency was classified as mild (CD4% of 30–35, 25–30, 20–25 and CD4 cell count of 350–499 for children ≤11 months, 12–35 months, 36–59 months or ≥5 years, respectively), advanced (CD4% of 25–29, 20–24, 15–19 and CD4 cell count of 200–349 for children ≤11 months, 12–35 months, 36–59 months, or ≥5 years, respectively) and severe (CD4% <25, <20, <15 and CD4 cell count <200/<15% for children ≤11 months, 12–35 months, 36–59 months or ≥5 years, respectively) according to the WHO 2006 thresholds.

relative hazards from the model are shown in Supplementary Table 2 (see online Supplementary Material for this table).

DISCUSSION

In this study, a notable proportion (29%) of HIV-positive children experienced clinical and/or immunological cART failure, with a crude incidence rate of 20.0 events per 100 person-years. Our findings appear to be in line with evidence from the literature referring to immunological failure [16–18]. Considering that virological failure tends to precede clinical and immunological failure, this figure could underestimate a greater impact of virological failure.

WHO clinical stage 4 and TB co-infection at cART initiation were significantly associated with treatment failure. Poor clinical status has been observed to negatively affect treatment response; in particular, malnutrition and chronic diarrhea independently increase the risk of treatment failure as much as baseline low immunity, high viral load (VL), and younger age [15–17]. As suggested by Hermans et al. [40, 41], TB co-infection may impair immune recovery after cART initiation in adults. In addition, poor adherence may occur as a result of high pill burden, and interaction with rifampicin may affect the bioavailability of HIV drugs, particularly for NVP and LPV/r [42]. Development of better options for TB co-treatment appears to be critical to prolong effectiveness of first-line regimens.

As expected, unconventional regimens were associated with treatment failure compared to triple cART [37]. Treatment failure was not different between PI-based and NNRTI-based regimens; however, the validity of this finding may be questionable considering that only a few children were receiving a PI-based regimen at the time of the study. Few randomized trials investigated the most effective first-line cART regimen in HIV-positive children. The P1060 trial [13, 23] showed an increased risk of virological failure in children (<3 years) on NPV-based cART, regardless of prevention of mother to child transmission (PMTCT) exposure; however, this was not confirmed by the PENPACT trial conducted in older children of high-income countries [24]. Due to the nature of our cohort's age and lack of reliable PMTCT exposure data, our observational retrospective findings are not comparable to those from either controlled trial.

Reasons for drug substitution were assessed to explore whether this was in response to treatment failure. However, over a 5-year period, only 4% of 202 patients who substituted cART switched to a second-line regimen due to treatment failure. Drug substitution occurred after a median time of 26.65 months, indicating a significant delay in switching to second-line despite the high rate of failure retrospectively observed in the cohort. Although reasons for substitution may have been misclassified, the small number of children switching due to treatment failure implies that a prolonged exposure to failing regimens may have occurred in these two settings. Several studies reported a low proportion of children on second-line cART in LMIC [19, 20]. Our switch rate appears even lower than those observed by Davies et al. [16] and by 2 other observational studies showing that around 14% children switched to second-line due to clinical and/or immunological failure [17, 18]. Reasons explaining the alarming gap between a recognized clinical and/or immunological failure and the initiation of a second-line cART were not well identified. In our program, we hypothesize that limited availability and costs of second-line drugs may be major barriers to second-line therapy. Furthermore, the tradeoff that clinicians are facing when considering the limited options for children failing first-line and the risk of maintaining them on a failing regimen can be very challenging and may result in further delays in switching to second-line cART. Underdiagnosis of treatment failure may also have contributed to the low rate of switching observed, as reasons for switching were collected retrospectively based on clinician report, leading to possible misclassification.

Determining when to switch to second-line cART is a critical decision in settings where virological monitoring is not available. Although evidence shows that VL is not essential to identify treatment failure [34], using clinical and immunological parameters leads to delays in switching to second-line therapy [17], resulting in longer exposure to failing regimens, which contributes to development of drug-resistant HIV strains [6]. In our study, reasons for delays to cART switching were not completely clarified; in particular, we were unable to understand if clinicians did not switch cART in children with recognized treatment failure or if clinical/immunological criteria were too complicated to recognize treatment failure. Earlier cART initiation and VL monitoring are currently recommended by WHO 2013 consolidated guidelines [3]. Based on our data, advanced disease and TB co-infection should be considered as warning signals requiring closer follow-up and counseling to improve treatment outcomes and prolong duration of first line therapy. Adherence to cART was found to be a poor indicator of treatment failure, maybe due to the low accuracy of self-reporting adherence monitoring.

About 26% (203/769) of patients substituted treatment with an overall incidence rate of 13.5 events per 100 person years and 95% of these were for causes other than treatment failure. This figure is consistent with previous

observational studies among HIV-positive children [17, 24, 40] living in LMIC. Toxicity/intolerance was one of the main reasons reported for substitution (18.3%), mostly related to AZT toxicity (12.4%), as reported in other studies [17, 38, 41]. Due to high prevalence of HIV/TB co-infection (88/769, 11.4%), drug interaction in TB/HIV co-treatment (25.7%) was another major reason to substitute drugs. Drug availability (17.3%) was another considerable reason, reflecting the importance of ensuring adequate and continuous supply of cART in settings where drug costs are still a major barrier for PLWH. Reasons for drug substitution were not classified prospectively but assessed from inspection of patient clinical charts, potentially leading to inaccurate classifications.

Higher rates of drug substitution were observed among children starting AZT-containing or EFV-based regimens. Increased drug substitution while on AZT is often the result of AZT-related anemia as well described previously [23, 31, 36]. AZT toxicity was more prevalent among the Mozambique cohort, where children were younger and malnutrition and/or more advanced WHO disease stages were observed, suggesting that AZT anemia may have been exacerbated. Despite the lack of more robust evidence, our findings suggest that AZT may not be the preferred NRTI to be used in these settings, particularly in younger children.

Further description of EFV substitution was not possible in this dataset due to the limited number of children receiving this drug, and we could not rule out specific EFV-related toxicity.

As previously mentioned, our results may be confounded by country-specific differences. Mozambique patients were younger, had a more advanced WHO stage, and a lower BMI z-score at cART initiation. These differences may reflect clinicians' preference in first-line treatment choice, accounting for the wider use of AZT and EFV in Uganda as much as for the increased choice of NVP-based regimen observed in children from Mozambique. Country-specific differences may potentially confound the relationships seen between cART regimen and treatment failure and drug substitution. In terms of follow-up visits, the Ugandan children were followed up much more frequently (monthly) than those in Mozambique (every 3 months). This difference between program performances may have provided further confounders, potentially influencing the trends observed in older children at lower risk of failure and the higher rate of drug substitution observed in infants.

In conclusion, our data reinforce the need for simplification of more effective clinical and immunological criteria for prompt recognition of cART treatment failure. Children presenting with advanced disease and TB co-infection should be targeted for closer and more sensitive monitoring of treatment response. This should be matched with a regular provision of appropriate antiretrovirals and with optimization of first-line drugs and treatment sequencing. Supply of new pediatric formulations for second-line regimens and drug optimization should be considered as critical milestones to allow scaling up of early cART and reduction of treatment failure in children.

Supplementary Data

Supplementary materials are available at the *Journal of The Pediatric Infectious Diseases Society* online (http://jpids.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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