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Plasma Efavirenz Exposure, Sex, and Age Predict Virological Response in HIV-Infected African Children

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Background: Owing to insufficient evidence in children, target plasma concentrations of efavirenz are based on studies in adults. Our analysis aimed to evaluate the pediatric therapeutic thresholds and characterize the determinants of virological suppression in African children.

Methods: We analyzed data from 128 African children (aged 1.7–13.5 years) treated with efavirenz, lamivudine, and one among abacavir, stavudine, or zidovudine, and followed up to 36 months. Individual pharmacokinetic (PK) measures [plasma concentration 12 hours after dose (C12h), plasma concentration 24 hours after dose (C24h), and area under the curve ($AUC_{0\text{-}24}$)] were estimated using population PK modeling. Cox multiple failure regression and multivariable fractional polynomials were used to investigate the risks of unsuppressed viral load associated with efavirenz exposure and other factors among 106 initially treatment-naive children, and likelihood profiling was used to identify the most predictive PK thresholds.

Results: The risk of viral load >100 copies per milliliter decreased by 42% for every 2-fold increase in efavirenz mid-dose concentration [95% confidence interval (CI): 23% to 57%; $P < 0.001$]. The most predictive PK thresholds for increased risk of unsuppressed viral load were C12h 1.12 mg/L [hazard ratio (HR): 6.14; 95% CI: 2.64 to 14.27], C24h 0.65 mg/L (HR: 6.57; 95% CI: 2.86 to 15.10), and $AUC_{0\text{-}24}$ 28 mg·h/L (HR: 5.77; 95% CI: 2.28 to 14.58). Children older than 8 years had a more than 10-fold increased risk of virological nonsuppression ($P = 0.005$); among children younger than 8 years, boys had a 5.31 times higher risk than girls ($P = 0.007$). Central nervous system adverse events were infrequently reported.

Conclusions: Our analysis suggests that the minimum target C24h and $AUC_{0\text{-}24}$ could be lowered in children. Our findings should be confirmed in a prospective pediatric trial.

Key Words: efavirenz, PK/PD, children

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INTRODUCTION

The nonnucleoside reverse transcriptase inhibitor efavirenz is recommended by the World Health Organization (WHO) as part of first-line treatment for HIV-infected children older than 3 years.[1] Owing to its high potency, long half-life, and availability of low-cost generic formulations, efavirenz continues to be one of the most widely used antiretrovirals in Africa and worldwide.[2] The mid-dose plasma concentration target of 1.0–4.0 mg/L derived from adult clinical monitoring data is customarily also applied to trough concentrations.[3,4] In adults, systemic exposure below that range is associated with

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virological failure and higher exposures with central nervous system (CNS) toxicities.[3,5,6] The same target range is used in children; however, rigorous analyses have not confirmed the optimal therapeutic range for this age group.[7–11]

The main objective of a pharmacokinetic/pharmacodynamic (PK/PD) analysis is to quantify the relationships between drug dose, exposure, and response, identifying factors affecting drug disposition and efficacy.[4] Although the high variability in efavirenz PK in children has been thoroughly studied,[12–15] analyses successfully relating observed drug exposures to treatment response and detecting other determinants of treatment failure are limited.[9,10,16] Factors affecting efavirenz effectiveness have often been investigated independently of drug concentrations with inconclusive findings across studies[8,9,17–20]; similarly, the effect of high efavirenz exposure on increased risk of CNS adverse events (AEs) is unconfirmed in children.[8,21–23]

The recent results of ENCORE1,[24,25] showing that the standard 600 mg efavirenz dose can be reduced to 400 mg daily without loss of efficacy in nonpregnant adults, have prompted discussions on the validity of the widely accepted efficacy thresholds of >1 mg/L, for a mid-dose interval or trough concentration,[25] and suggest that the target range used for children should also be reevaluated. Our analysis therefore aimed to characterize associations between systemic exposure to efavirenz and risk of virological nonsuppression and CNS AEs over the longer term, to identify factors affecting virological nonsuppression independently of systemic exposure, and to validate the lower boundary of the therapeutic range for efavirenz in African children.

METHODS

Population and Study Design

As described previously,[26] the Children with HIV in Africa–Pharmacokinetics and Adherence/Acceptability of Simple antiretroviral regimens study enrolled HIV-infected antiretroviral therapy (ART)-naive and ART-experienced children 13 years or younger in 4 sites in Uganda and Zambia. Of 478 participants, 128 received efavirenz and lamivudine combined with abacavir, stavudine, or zidovudine. Children switched to boosted protease inhibitor–based second-line ART for clinical or immunological failure following WHO 2010 guidelines. Samples for PK analysis were taken at week 6, week 36, and every 24 weeks thereafter. Efavirenz PK was described previously.[27] Viral load (VL) was measured retrospectively in stored plasma samples taken at enrollment and weeks 48, 96, and 144, and at weeks 36, 60, 84, 108, and 132 when PK samples were taken. An undetectable VL was defined as <100 copies per milliliter, the lower limit of detection, because many samples had to be diluted owing to low volumes.

Statistical Analysis

Empirical Bayesian estimates for the individual parameters from the previously developed population PK (POP-PK) model were used to estimate steady-state mid-dose efavirenz concentrations (C12h, defined as plasma concentration 12 hours after dose), trough concentrations (C24h, plasma concentration 24 hours after dose), and $AUC_{0\text{-}24}$ (area under the curve) for each child at each included timepoint.[27]

Children followed for <48 weeks were excluded from all analyses. For a preliminary analysis, VL response was categorized as suppressed (<100 copies/mL achieved within 48 weeks of treatment initiation and maintained throughout the study), single rebound (<100 copies/mL within 48 weeks and a single viral rebound >100 copies/mL), multiple rebounds (<100 copies/mL within 48 weeks and multiple viral rebounds), and never suppressed <100 copies per milliliter. Treatment-experienced children who were virologically suppressed at study enrollment were analyzed separately. As multiple PK exposures were available for each child, the geometric mean exposure value (derived from all PK visits) for each child was compared between groups using Kruskal–Wallis and rank sum tests. Categorical factors were compared between groups using Fisher exact test.

The effects of PK on virological nonsuppression (>100 copies/mL) were then estimated using Cox proportional hazards regression models (Andersen–Gill repeated outcomes framework) with Efron approximation in R (survival package),[28–31] including only VLs measured on PK sampling days from week 36 onward in children who were treatment-naive at enrollment. Samples taken before initial viral suppression were excluded, unless children never suppressed during the study. Each time interval ran from the preceding to current VL (classified as suppressed vs nonsuppressed "event"), and the estimated PK parameters at the current VL were applied to the whole time interval. Nonlinearity in effect of PK exposures was explored visually using smoothed splines, and tested using fractional polynomials (using Stata 14.0 mfp, StataCorp LP, College Station, TX).[32] The best-fitting (lowest Akaike Information Criterion) dichotomous threshold was identified by profile likelihood. Because PK parameters were estimated and not observed, we used a resimulation approach to assess the impact of unobserved variability on selection of the dichotomized threshold. The original data set was resimulated 500 times introducing a normally distributed random error on each of the exposure parameters, set to the unexplained residual variability from the POP-PK model (additive error 0.101 mg/L, proportional error 0.0672).[27] The results were used to derive 95% confidence interval (CI) for the threshold (2.5th and 97.5th percentile of distribution of most predictive cutoffs from 500 runs).

For each PK exposure threshold identified in this study and the previously proposed efficacy thresholds, we calculated the sensitivity (proportion of samples correctly predicted as notsuppressed), specificity (proportion of samples correctly predicted as suppressed), accuracy (overall proportion of correctly predicted samples), positive predictive value (proportion of samples with exposure below the threshold not suppressed), and negative predictive value (proportion of samples with exposure above the threshold that were suppressed).

Finally, we used backward elimination (exit $P = 0.05$, retaining all levels of categorical factors where any were $P < 0.05$) to consider the additional independent effects of covariates on nonsuppression with associations ($P < 0.2$) in univariable models. Categorical covariates included nucleoside reverse transcriptase inhibitor backbone (abacavir, zidovudine,

stavudine), sex, clinical site, mother as primary carer, and self-reported missing doses in previous 4 weeks. Continuous variables included pre-ART VL, CD4% pre-ART and at the time of PK/VL measurement, age, weight-for-age Z-score,[33] height-for-age Z-score,[33] and Medication Event Monitoring System (MEMS) adherence [proportion of days without drug intake based on MEMS cap container openings in the interval between previous and current measurement (truncated at a lower limit of 0.5); the only covariate with incomplete information was adherence; where no data was available for current interval the previous MEMS adherence was carried forward, and if no MEMS adherence data were available for the child (N = 19) we imputed the median of all treatment-naïve patients]. Only 1 child had concurrent coadministration of antituberculosis drugs, so this factor was not considered. Nonlinear effects in continuous variables were included using fractional polynomials (Stata mfp). Interactions between factors included in the final model were investigated and included if $P < 0.05$. The impact on PK exposure of metabolic status based on *CY2B6* 516 GT|983 TC single nucleotide polymorphisms[34] was then investigated by adding this factor into the final model.

CNS Adverse Events

Specific CNS toxicities relating to cognitive or motoric functions were solicited at every follow-up visit (concentration, vivid dreams/nightmares, sleepiness/sleepwalking, waking at night, difficulty waking in the morning, dizziness) and graded between 1 and 3 (mild to severe). Incidence of CNS AEs was compared between groups using Fisher exact test.

RESULTS

In total, 128 children (14 being treatment-experienced) received efavirenz in CHAPAS-3 and contributed a total of 1482 PK measurements from 570 PK visits, 345 with paired VL measurements. Five children with <48 week follow-up were excluded from all analyses, and a further 3 children with no paired PK-VL measurements were excluded from the Cox model. Table 1 shows child characteristics and model-derived PK parameters in each suppression group. Sixty-seven percent of children (n = 73) who were treatment-naive at enrollment achieved and maintained viral suppression <100 copies per milliliter, 17% (n = 19) had a single episode of

TABLE 1. Demographic Characteristics and Model-Derived PK Parameters in Different Suppression Groups

	Treatment-Naive at Enrollment					Treatment-Experienced at Enrollment	
	Suppressed	Single Rebound	Multiple Rebound	Never Suppressed			
No. children	73	19	10	7	*P**	14	*P*†
Baseline							
Age (yrs)	4.3 (3.5–4.7)	3.9 (3.6–4.5)	3.5 (3.3–3.8)	3.5 (3.2–8.5)	0.208	7.3 (5.7–8.5)	<0.001
Weight (kg)	14.0 (12.4–16.0)	14.5 (13.5–16.0)	13.4 (12.8–15.7)	12.3 (12.0–17.5)	0.513	20.1 (19.3–22.6)	<0.001
CD4% (%)	18.5 (11.0–24.0)	17.2 (7.3–22.1)	19.5 (15.5–24.5)	19.5 (10.3–19.5)	0.565	35.6 (31.4–37.8)	<0.001
CD4 (cells/mL)	707 (497–977)	648 (204–904)	684 (477–1047)	618 (201–913)	0.429	915 (807–1249)	<0.001
Viral load (copies/mL)	172,165 (63,250–338,685)	93,160 (23,415–218,830)	175,390 (130,080–606,275)	149,080 (69,065–354,885)	0.268	<100	<0.001
Sex (M/F)	29/44	10/9	8/2	4/3	0.133	9/5	0.091
Metabolic subgroup‡							
EM	24	7	1	2	0.073	5	0.173
IM	28	6	8	3		6	
SM	21	6	—	2		3	
USM	—	—	1	—		—	
NRTI							
Stavudine	23	7	5	1	0.738	5	0.908
Zidovudine	26	6	2	4		4	
Abacavir	24	6	3	2		5	
PK measure							
$AUC_{0\text{-}24}$ (mg·h/L)	57.1 (37.1–101.4)	57.8 (45.9–121.8)	46.6 (42.8–78.0)	36.8 (13.6–74.0)	0.360	77.7 (58.5–114.2)	0.142
C12h (mg/L)	2.25 (1.43–4.11)	2.22 (1.77–4.96)	1.93 (1.65–3.08)	1.40 (0.5–2.83)	0.367	3.12 (2.38–4.43)	0.155
C24h (mg/L)	1.54 (0.95–3.15)	1.43 (1.19–3.69)	1.24 (1.01–1.77)	0.76 (0.31–1.82)	0.223	2.27 (1.630–3.53)	0.123
Cmax (mg/L)	4.20 (3.03–6.28)	4.42 (3.22–6.59)	3.81 (3.24–6.99)	3.50 (1.07–5.62)	0.606	5.33 (4.70–6.66)	0.159
CL (L/h)	5.6 (3.2–7.7)	5.6 (2.4–7.3)	6.2 (5.2–7.6)	9.1 (5.9–9.2)	0.382	6.5 (4.3–8.3)	0.479
Adherence (MEMS caps)§	1.00 (0.97–1.00)	0.98 (0.95–1.00)	0.98 (0.91–0.99)	0.87 (0.69–0.95)	0.010	0.97 (0.91–1.00)	<0.001

Presented values are number or median (IQR).
*Kruskal–Wallis or Fisher exact test comparing 4 groups of originally treatment-naive children only.
†Kruskal–Wallis or Fisher exact test comparing 5 groups including children who were treatment-experienced at enrollment.
‡EM (extensive metabolizers), 516 GG|983 TT; IM (intermediate metabolizers), 516 GG|983 TC or 516 GT|983 TT, SM (slow metabolizers), 516 TT|983 TT or 516 GT|983 TC; USM (ultraslow metabolizers), 516 GG|983 CC.
s§Data from 104 patients (91 treatment-naive and 13 treatment-experienced at enrollment).
F, female; IQR, interquartile range; M, male; MEMS, Medication Event Monitoring System; NRTI, nucleoside reverse transcriptase inhibitor.

viral rebound, while 15% (n = 17) had multiple viral rebounds or never suppressed. There were no statistically significant differences in baseline (pre-ART) demographic characteristics or geometric mean PK parameters across study follow-up between these 4 groups. However, there was a trend to lower average exposures and higher average clearance among treatment-naive children who never suppressed, compared with children who achieved and sustained viral suppression (pairwise rank sum: C12h $P = 0.11$, C24h $P = 0.07$, AUC $P = 0.12$, clearance $P = 0.08$). Average adherence was also significantly lower in those who never suppressed ($P = 0.004$ vs sustained suppression), whereas there was no difference in demographics between these 2 groups.

Of 14 children who were treatment-experienced (and virologically suppressed) at enrollment, 1 had a single episode of viral rebound, the rest remained suppressed throughout follow-up. Children who were treatment-experienced at enrollment differed significantly from the treatment-naive children in baseline characteristics and had higher geometric mean efavirenz exposures (all $P < 0.05$ vs naive children combined) but no difference in average clearance ($P = 0.63$). Average adherence was marginally lower in treatment-experienced compared with the treatment-naive patients (0.97 vs 1.00, $P = 0.006$).

Hazard of Virological Nonsuppression

Repeated measures Cox proportional hazards regression models fitted to 345 matched PK-VL samples from 106 treatment-naive children indicated that the risk of virological nonsuppression increased approximately uniformly with each fold-change in PK exposures (ie, a log transform of PK exposure) (Table 2).

Profile likelihood identified thresholds of 1.12 mg/L (95% CI from resimulations 0.47 to 1.56 mg/L) for C12h, 0.65 mg/L (95% CI: 0.25 to 1.27) for C24h, and 28 mg·h/L (95% CI: 20.47 to 32.22) for $AUC_{0\text{-}24}$ as the best dichotomized thresholds for predicting virological suppression (see Supplemental Digital Content, http://links.lww.com/QAI/A819). For AUC, the model including log exposure was superior, whereas for C12h and C24h, a dichotomized threshold provided a better model fit, but these margins were relatively small (Table 2).

Multivariate Analysis

The 3 PK exposures were highly correlated (Spearman rho >0.98), which could be expected because they were derived from the same POP-PK model. We therefore only considered C12h in multivariable models. The only other factors associated ($P < 0.2$) with virological nonsuppression in univariate analyses were sex, site, current age, and current weight-for-age Z-score. However, only C12h, sex, site, and current age were independent predictors (selected using backward elimination). There was a significant interaction between sex and age ($P = 0.01$), ie, age was an effect modifier for sex. To represent this interaction, we dichotomized age at 8 years (based on univariate profile likelihood as for PK exposures). Adjusting for other factors, the hazard of virological nonsuppression for boys <8 years was 5 times greater than that for girls of similar age (Table 3). Older children had increased risk of virological nonsuppression compared with younger children, but there was no evidence of a difference between boys and girls >8 years ($P = 0.76$). The hazard of virological nonsuppression was significantly higher in the smallest site, which contributed only 5 children. There was marginal evidence that poorer MEMS adherence independently increased the hazard of virological nonsuppression ($P = 0.065$; effects of other factors, including C12h, were similar to Table 3). The remaining factors, including metabolizer status ($P = 0.27$), did not have an effect on viral nonsuppression ($P > 0.1$).

TABLE 2. Univariable Cox Proportional Hazards Regression Models for C12h, C24h, and AUC, With Lowest AIC Values Indicating the Models Best Describing the Association With Viral Nonsuppression

Pharmacokinetic parameter	Change in Risk Per Unit Increase in Absolute Exposure	Change in Risk Per Doubling of Exposure (Per Unit Increase in Log_2 Transformed Exposure)	Change in Risk Change at Threshold for Dichotomized Exposure Variables (Supplement 1)
C12h (mg/L)			
HR (95% CI)	0.87 (0.69 to 1.10)	0.58 (0.43 to 0.77)	6.14 (2.64 to 14.27) (vs C12h >1.12 mg/L)
P	0.241	<0.0001	<0.0001
AIC	324.11	305.76	304.55
C24h (mg/L)			
HR (95% CI)	0.86 (0.67 to 1.11)	0.60 (0.46 to 0.78)	6.57 (2.86 to 15.10) (vs. C24h >0.65 mg/L)
P	0.246	<0.0001	<0.0001
AIC	324.67	304.18	302.82
AUC (mg·h/L)			
HR (95% CI)	0.9941 (0.9843 to 1.0040)	0.57 (0.42 to 0.76)	5.77 (2.28 to 14.58) (vs. AUC >28 mg·h/L)
P	0.247	<0.0001	<0.0001
AIC	324.04	305.70	307.74

Log transform was the best-fitting fractional polynomial for C24h; for C12h and AUC the best-fitting transform was inverse square root. However, the difference in AIC compared with log transform was very small in both cases (+0.47 and +0.97) and so the log transform is presented above for comparability with C24h and ease of interpretation.

AIC, Akaike Information Criterion; HR, hazard ratio.

TABLE 3. Univariate and Multivariate Predictors of Virological Suppression

	Univariate		Final Multivariate Model	
Factor	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
C12h (per doubling)	0.58 (0.43 to 0.77)	<0.001	0.61 (0.50 to 0.76)	<0.001
Sex: male vs female	2.77 (1.01 to 7.64)	0.048	(See interaction below)	
Current age (reference <8 yrs)	5.45 (1.85 to 16.06)	0.002	(See interaction below)	
Sex and age (reference girl <8 yrs)	Boy <8 yr: 6.14 (2.01 to 18.77)	0.001	Boy <8 yr: 5.31 (1.58 to 17.82)	0.007
	Girl >8 yr: 16.63 (4.05 to 68.37)	<0.001	Girl >8 yr: 15.82 (2.97 to 84.27)	0.001
	Boy >8 yr: 25.50 (3.37 to 193.13)	0.002	Boy >8 yr: 12.47 (1.31 to 119.08)	0.028
Site (reference S1)	S2: 0.22 (0.06 to 0.77)	0.018	S2: 0.73 (0.18 to 2.88)	0.653
	S3: 0.39 (0.11 to 1.38)	0.146	S3: 1.04 (0.23 to 4.82)	0.956
	S4: 2.48 (0.69 to 8.99)	0.166	S4: 4.96 (1.38 to 17.79)	0.014
WAZ (per unit higher)	0.66 (0.49 to 0.88)	0.005	—	

As the final multivariable model identified a significant interaction between age and sex, this interaction is also presented unadjusted for other factors in the univariable column. Final model selected using backward elimination, see methods. Interaction between continuous age and sex ($P = 0.01$) dichotomized at the optimal age threshold for presentation.
HR, hazard ratio; WAZ, weight-for-age Z-score.

CNS Adverse Events

Despite being solicited at every follow-up visit, only 18 CNS AEs were reported in 11 children (3 problems with concentration, 4 vivid dreams, 2 sleep walking, 2 difficulties waking up in the mornings, 3 waking up at night, 4 dizziness; all but one graded mild). These 11 children included 5 slow, 4 intermediate, and 2 extensive metabolizers (exact $P = 0.41$). Nine children reported one of these AEs <24 weeks after treatment initiation. Only 2 children reported AEs on repeated occasions (both slow metabolizers), of which only 1 had a paired PK sample: plasma efavirenz 4 hours after dose was 45 mg/L, but the child was incorrectly receiving 600 mg instead of a 400 mg dose.

DISCUSSION

We observed that efavirenz concentrations were related to virological nonsuppression in African children in a nonlinear manner, a 2-fold increase in efavirenz exposure decreased the risk of virological nonsuppression by over 40%. Some previous studies failed to detect a similar association,[7,8,35] which could be due to a number of reasons:

TABLE 4. Comparison of Efavirenz Exposure Targets and Predictors of Virological Outcome in Pediatric Studies

		Predictors of Virologic Failure				
Reference	**Derived PK Targets**	**PK**	**Covariates**	**n**	**Method**	**VL Target (Copies/mL)**
Starr et al[21]*	AUC = 60–120 mg·h/mL	Not analyzed	Uni: (A)† $\log_2$ bCD4%, WAZ, bVL/(B)† WAZ, bVL	57	Cox	400 (A)†
			Multi: (A)† WAZ, bVL/(B)† bVL			50 (B)†
Brundage et al[16]	AUC >59 mg·h/mL	AUC	Uni: IPAM, bVL, bCD4%, WAZ	50	Cox, TSSA	400
			Multi: IPAM, bVL, AUC			
Hirt et al[10]	C_{min} >1.1 mg/L	C_{min}, AUC	Not analyzed	48	Fisher exact test	300
	AUC >51 mg·h/L					
Fletcher et al[9]	AUC >49 mg·h/mL	AUC	Not analyzed	50	Logistic regression	400
Janssens et al[20]	Not analyzed		Uni: Orphan status, male gender	212	Logistic regression	400
			Multi: Orphan status			
Kamaya et al[18]‡	Not analyzed		Uni: male gender, bCD4% <5%	250	Logistic regression	400
			Multi: male gender, bCD4% <5%			
Jittamala et al[19]‡	Not analyzed		Uni: male gender, age, adherence	202	Cox	50
			Multi: none			
Bienczak et al (this analysis)	C_{12h} >1.12 mg/L	C_{12h}, C_{min}, AUC	Uni: male gender, age <8 years, site, WAZ	118	Cox	100
	C_{min} >0.65 mg/L		Multi: male gender, age <8 yrs			
	AUC >28 mg·h/L					

*Target derived based on adult data.
†Two efficacy cutoffs used: (A) 400 copies per milliliter, (B) 50 copies per milliliter; bCD4%, baseline (pre-ART) CD4 percentage.
‡Patients treated with nevirapine or efavirenz; presented results relate to efavirenz only.
bVL, baseline (pre-ART) viral load; Cox, Cox proportional hazards regression; IPAM, integrated pharmacokinetic adherence measure; multi, multivariate analysis; TSSA, tree-structured survival analysis; Uni, univariate analysis; VL, viral load; WAZ, weight-for-age Z-score.

their follow-up time was short, they had only a single outcome at one time point, they were underpowered to characterize the PK/PD relationship, or tried to simplify it by a linearization.[7,8,21] To avoid such limitations our study analyzed a unique set of matched PK/VL longitudinal data using Cox multiple failure regression, allowing for repeated within-child measurements, similar to Van Leth et al[36] and Brundage et al.[16] This approach enabled us to identify the most predictive dichotomous threshold related to increased risk of VL >100 copies per milliliter for each PK parameter using profile likelihood, allowing for uncertainty in estimated PK exposures by a resampling approach.

Comparing our findings with previously proposed cutoffs (Table 5), the 1.12 mg/L threshold we obtained for C12 h does not differ markedly in sensitivity, specificity, or negative predictive power from the 1.0 mg/L value proposed by Marzolini et al.[3] However, our cutoffs for C24h and $AUC_{0\text{-}24}$ (0.65 mg/L and 28 mg·h/L, respectively) are lower than previously derived targets,[3,9,10,16,36] and substantially improved specificity, accuracy and positive predictive power, while maintaining a negative predictive power comparable with previously suggested therapeutic thresholds. Although our revised cutoffs require independent validation in a prospective pediatric trial, they were determined from the PK/PD relationship rather than using arbitrary percentiles of PK exposure distribution.

The results of the ENCORE1 study question the validity of a 1 mg/L efficacy threshold in adults,[25] but owing to low failure rates in the study the authors failed to detect a significant relationship between efavirenz exposure and the virological outcome.[35] Owing to design, analytical, and population differences, our study was able to define efavirenz exposure thresholds associated with increased risks of virological nonsuppression. Our findings should not be extrapolated to adults. Efavirenz clearance in children is relatively higher than that in adults, which could affect the suggested cutoffs, especially for C24h and AUC. Furthermore, other differences in PK or pathophysiology between those populations cannot be excluded, and the companion drugs used in the pediatric antiretroviral regimens are different from those used in adults. Although the threshold we identified for C12h is not markedly different from 1 mg/mL, our findings do not support dose reduction in children. In our previous analysis, we reported that the average exposures across pediatric weight bands dosed according to the current WHO recommendations were above that cutoff.[27] However, the average exposures were significantly affected by CYP2B6 516 G>T|983T>C genotype, and individuals wild type for those polymorphisms are at risk of subtherapeutic exposures. The results of the current analysis support modifications of the pediatric dosing recommendations based on individual metabolic status.

Among younger children (<8 years), we found a higher risk of virological nonsuppression in boys. Older children (>8 years) has similarly high risk of viral nonsuppression in both girls and boys. This phenomenon could arise from differences in treatment adherence by age, because similar effects were observed after adjusting for MEMS adherence. Although the latter is an imperfect measure of adherence, numerous studies have showed that treatment adherence declines with decreasing levels of parental supervision over daily drug intake in older children and adolescents.[37,38] It is less likely that different treatment adherence explains differences between younger boys and girls, in whom caregivers supervise medication intake. Similar effects of male sex were detected in pediatric studies by Janssens et al,[20] Kamaya et al,[18] and Jittamala et al[19] (Table 4).

Adherence measures are cumulative over time since the last visit, whereas PK exposures may be influenced by enhanced pill-taking immediately before clinic visits. In our analysis, children who never suppressed had lower average adherence scores and a trend to lower systemic exposures than those who suppressed; a similar trend was identified in the multivariate analysis. In keeping with our findings, Brundage et al[39] showed that the effect of adherence on the hazard of virological failure was independent of efavirenz exposure.

Children who were treatment-experienced at enrollment were excluded from our main analysis for several reasons.

Table 5. Comparison of Previously Published Treatment Targets for Efavirenz Concentrations and AUC and Most Predictive Thresholds Derived in This Analysis

	C12h (mg/L)				C24h (mg/L)				AUC (mg·h/L)					
Threshold	1.0[3]		1.12		1.0[3]		0.65		49[9]		60[22]		28	
HR	6.36		6.14		3.96		6.57		3.16		3.84		5.77	
95% CI	2.53 to 15.96		2.64 to 14.27		1.73 to 9.03		2.86 to 15.10		1.39 to 7.16		1.56 to 9.44		2.28 to 14.58	
AIC	305.35		304.55		315.07		302.82		319.86		318.12		307.74	
Samples not sup/sup (n)	<T	>T	<T	>T	<T	>T	<T	>T	<T	>T	<T	>T	<T	>T
	17/27	21/281	19/34	19/274	21/66	17/242	19/32	19/276	24/101	14/207	30/153	8/155	18/32	20/276
Sensitivity, %	44.7		50.0		55.3		50.0		63.2		78.9		44.74	
Specificity, %	91.2		88.9		78.5		89.6		67.1		50.2		90.23	
Accuracy, %	86.1		84.7		76.0		85.3		66.8		53.5		85.2	
Positive predictive value, %	38.6		35.8		24.1		37.3		19.2		16.4		36.17	
Negative predictive value, %	93.0		93.5		93.4		93.5		95.1		95.1		92.95	

In gray: cutoffs proposed by this analysis, in white: previously published cutoffs.
AIC, Akaike information criterion; HR, hazard ratio; sup, suppressed; T, cutoff target.

Inclusion criteria required these children to have been on effective antiretroviral treatment for >2 years and have suppressed VL. It is possible that they therefore had better adherence or were infected with HIV strains free of non-nucleoside reverse transcriptase inhibitor resistance mutations (no pre-ART genotypes were available). They also differed significantly from treatment-naive children by being older and healthier. Interestingly, their PK exposures tended to be higher, supporting a selection effect whereby those with optimal viral suppression are more likely to have higher exposure. All the matched PK/VL samples for this group of children were suppressed, and so we could not estimate the subsequent hazard of virological nonsuppression.

Our study has several limitations. Most important is the risk of overfitting the current data when estimating a dichotomized efficacy threshold, with lower external generalizability, which we were unable to test in a validation data set. The proposed thresholds should also be interpreted in terms of treatment effectiveness in the clinical setting of our study population; their value may be lower in a setting of complete treatment adherence. Adherence in our study was measured only in certain time periods, and participants did not use MEMS caps throughout the trial. This intermittent assessment could introduce error into adherence measurement, subsequently affecting the estimated effect of adherence on the risk of nonsuppression. Moreover, the wide CIs for efavirenz exposure thresholds predicting a detectable VL show that larger studies are needed to define thresholds more precisely. We had no VL data between treatment start and week 36 and therefore could not examine factors affecting time to first suppression, or the impact of PK parameters on VL decline. Furthermore, VL was measured on average only every 24 weeks, so our analysis assumes that no viral rebounds occurred between scheduled measurements.

Despite major concerns, very little CNS toxicity was reported in these predominantly younger children, although this may be more important in adolescents.[40] The relationship between high efavirenz exposures and CNS side effects detected in adults still remains unclear in children.[8,21–23]

Last, antiretroviral therapy consists of a combination of drugs and its efficacy depends on all the components of the tested regimen. Children in CHAPAS-3 were treated with efavirenz and a nucleoside reverse transcriptase inhibitor backbone consisting of lamivudine combined with either abacavir, stavudine, or zidovudine.[26] Our findings might not be generalizable to different drug combinations, for example, those including more effective companion drugs such as tenofovir, although this is still rarely used in children because of concerns about its impact on growth.

CONCLUSIONS

Efavirenz exposure predicts virological outcome, independently of other factors, including adherence, with every 2-fold increase in efavirenz concentration reducing the hazard of nonsuppression by about 40%. The widely accepted lower therapeutic threshold of 1 mg/L for mid-dose concentrations derived in adults is applicable in children, but the cutoffs for trough concentration and $AUC_{0\text{-}24}$ could be lowered to 0.65 mg/L and 28 mg·h/L, respectively. Our findings should be confirmed in a prospective pediatric trial.

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