

Determinants of virological outcome and adverse events in African children treated with paediatric nevirapine fixed-dose-combination tablets

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Background: Nevirapine is the only nonnucleoside reverse transcriptase inhibitor currently available as a paediatric fixed-dose-combination tablet and is widely used in African children. Nonetheless, the number of investigations into pharmacokinetic determinants of virological suppression in African children is limited, and the predictive power of the current therapeutic range was never evaluated in this population, thereby limiting treatment optimization.

Methods: We analysed data from 322 African children (aged 0.3–13 years) treated with nevirapine, lamivudine, and either abacavir, stavudine, or zidovudine, and followed up to 144 weeks. Nevirapine trough concentration (C_{\min}) and other factors were tested for associations with viral load more than 100 copies/ml and transaminase increases more than grade 1 using proportional hazard and logistic models in 219 initially antiretroviral treatment (ART)-naive children.

Results: Pre-ART viral load, adherence, and nevirapine C_{\min} were associated with viral load nonsuppression [hazard ratio = 2.08 (95% confidence interval (CI): 1.50–2.90, $P < 0.001$) for 10-fold higher pre-ART viral load, hazard ratio = 0.78 (95% CI: 0.68–0.90, $P < 0.001$) for 10% improvement in adherence, and hazard ratio = 0.94 (95% CI: 0.90–0.99, $P = 0.014$) for a 1 mg/l increase in nevirapine C_{\min}]. There were additional effects of pre-ART CD4⁺ cell percentage and clinical site. The risk of virological nonsuppression decreased with increasing nevirapine C_{\min} , and there was no clear C_{\min} threshold predictive of virological nonsuppression. Transient transaminase elevations more than grade 1 were associated with high C_{\min} (>12.4 mg/l), hazard ratio = 5.18 (95% CI 1.95–13.80, $P < 0.001$).

Conclusion: Treatment initiation at lower pre-ART viral load and higher pre-ART CD4⁺ cell percentage, increased adherence, and maintaining average C_{\min} higher than current target could improve virological suppression of African children treated with nevirapine without increasing toxicity.

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Introduction

Fixed-dose combination (FDC) formulations have considerably improved access to antiretroviral treatment (ART) through decreased cost and improved feasibility, especially in sub-Saharan Africa [1]. Currently available paediatric-dispersible FDCs are limited to combinations of a nonnucleoside reverse transcriptase inhibitor (NNRTI), nevirapine, with two nucleoside-reverse transcriptase inhibitors (NRTIs), and are widely used in children in low-income countries [2].

Nevirapine pharmacokinetics exhibits high variability, attributed in part to single nucleotide polymorphisms (SNPs) of cytochrome *P450 2B6* (*CYP2B6*), which encode an important metabolic pathway for this drug [3–5]. In adults, low nevirapine concentrations have been associated with increased risk of virological failure [6–8] and high exposures with increased risk of skin rashes [9–11] and hepatotoxicity [7,12]. Based on the concentration–response relationship, a therapeutic range of 3–8 mg/l has been suggested for nevirapine therapeutic drug monitoring [13]. However, several studies failed to confirm these associations [4,14,15], and low incidences of nevirapine-related adverse events have been reported in low-income settings [16] and in African children [17,18]. Despite widespread use, few studies have investigated the pharmacokinetic determinants of efficacy of nevirapine-based regimens in children. The predictive power of the suggested targets has also never been thoroughly investigated in black Africans or in children. Whether these pharmacokinetic targets should be universally applied across populations was recently questioned [4].

Our aim was therefore to investigate associations between nevirapine trough concentrations (C_{\min}) and long-term virological outcomes and adverse events in African children and establish if any other factors predict treatment outcome after adjusting for drug exposures, allowing treatment optimization.

Methods

Population and study design

The Children with HIV in Africa – Pharmacokinetics and Adherence/Acceptability of Simple Antiretroviral Regimens (CHAPAS-3) trial enrolled HIV-infected ART-naive and ART-experienced (>2 years ART with viral load <50 copies/ml at screening) children aged 0.3–13 years from four sites in Uganda and Zambia [19], treated following WHO 2010 guidelines [20] with an NNRTI (nevirapine or efavirenz) and two NRTIs (lamivudine and randomized abacavir, stavudine, or zidovudine). Nevirapine was coformulated with companion NRTIs in paediatric FDCs provided by Cipla (Mumbai,

Maharashtra, India) [19]. Children on nevirapine were switched to efavirenz-based ART if aged more than 3 years and diagnosed with tuberculosis (TB) or experienced nevirapine-related adverse events, or to boosted protease inhibitor-based ART if less than 3 years with these events or for clinical or immunological failure (or if efavirenz-intolerant). Samples for pharmacokinetics analysis (described previously) [5] were taken at weeks 6, 36, and every 24 weeks thereafter. Viral load was assayed retrospectively on stored plasma taken at enrolment and weeks 48, 96, 132, or 144.

Statistical analysis

A previously developed model describing the steady-state population pharmacokinetics of nevirapine [5] was used to derive empirical Bayesian estimates for each child at each pharmacokinetic visit for clearance, C_{\min} (evening trough concentration), C_{\max} (maximum concentration), and AUC_{0-24} (area under the curve). Due to diurnal variability in clearance [5], this analysis included measurements relating to daytime exposures only.

The primary efficacy outcome was viral load more than 100 copies/ml (the limit of detection as many samples had to be diluted due to low volumes). For descriptive analysis, response was categorized as suppressed (<100 copies/ml within 48 weeks of treatment initiation, maintained throughout follow-up), slow suppression (<100 copies/ml achieved after 48 weeks but maintained throughout subsequent follow-up), rebounded (<100 copies/ml within 48 weeks but viral load >100 copies/ml at single or multiple visits thereafter), and never suppressed (viral load never <100 copies/ml). ART-experienced children (all viral load <50 copies/ml at enrolment) were analysed separately from ART-naive children initiating treatment at enrolment. As multiple pharmacokinetic exposures were available for each child, geometric means of pharmacokinetics parameters across follow-up within each individual, and interindividual variability (expressed as coefficient of variation [21]) for C_{\min} and AUC_{0-24} were compared between groups using Kruskal–Wallis and rank-sum tests. Categorical factors were compared between groups using Fisher's exact test.

The effect of nevirapine C_{\min} on the risk of virological nonsuppression (>100 copies/ml) in the subset of ART-naive children only was estimated using Cox proportional hazards regression models (Andersen–Gill repeated outcomes framework) with Efron approximation in R (survival package) [22–25]. Viral loads were matched with the model estimated C_{\min} from the closest sampling visit preceding each viral load. Each time interval ran from the preceding to the current viral load (classified as suppressed vs nonsuppressed 'event'), and the matched C_{\min} was applied to the whole time interval. Nonlinearity in the effect of C_{\min} was explored visually using smoothed splines and tested using fractional polynomials (Stata mfp cox; Stata Statistical Software: Release 14. StataCorp. LP,

College Station, Texas, USA) [26]. As Cox regression does not provide estimates of the absolute probability of suppression, we estimated this using a mixed-effects repeated measures logistic model (Stata mfp logistic; StataCorp. LP) [26]. The best-fitting dichotomous threshold for nevirapine C_{\min} in the Cox model was identified by profile likelihood, as described previously for efavirenz [27]. Following the same method, we conducted simulations introducing unexplained residual variability on predicted concentrations from the population pharmacokinetics model (additive error 0.32 mg/l, proportional error 5.26%) [5] to derive 95% confidence intervals (CIs) for this threshold (2.5th and 97.5th percentile of most predictive cut-offs from 500 simulations). The sensitivity, specificity, accuracy, and positive and negative predictive values of the identified threshold for viral load suppression were compared with those of the 10th, 25th, and 50th percentiles of estimated nevirapine C_{\min} in this study and cut-offs proposed in the literature [6,7,13].

Finally, we used backwards elimination (exit $P=0.05$, retaining all levels of categorical factors where $P<0.05$) to consider the additional independent effects on nonsuppression of factors with associations ($P<0.2$) in univariate models. Categorical covariates included NRTI-backbone (abacavir, zidovudine, or stavudine), sex, clinical site, exposure to ART in children and/or mothers in prevention of mother-to-child transmission [pMTCT (regimens listed in footnote to Table 1)], metabolizer status (MET) based on *CYP2B6* 516G>T|983T>C SNP (extensive metabolizers – 516GG|983TT; intermediate metabolizers – 516GG|983TC or 516GT|983TT; slow metabolizers – 516TT|983TT or 516GT|983TC; and ultraslow metabolizers – 516GG|983CC) [5], mother as primary carer, and self-reported missing of any ART doses in previous 4 weeks. Continuous variables included baseline (pre-ART) viral load (bVL) and CD4⁺ cell percentage (bCD4⁺ cell percentage), truncated at 50% to avoid undue influence of outliers), current age, weight-for-age Z-score (WAZ) [28], height-for-age Z-score (HAZ) [28], and adherence [percentage of doses taken based on Medication Event Monitoring Systems (MEMS)-cap container openings in the interval between previous and current viral load (truncated to a lower limit of 0.5)]. The only factor with incomplete information was adherence; when no data were available for current interval, the preceding interval's value was carried forward. If no prior MEMS data were available ($n=21$), we imputed the median value for all ART-naive individuals. Nonlinear effects in continuous variables were explored as described above for C_{\min} . Interactions between factors included in the final model were investigated and included if P was less than 0.05.

Adverse events

Adverse events considered to be nevirapine-related were hypersensitivity reactions [HSR, including Stevens–

Johnson Syndrome (SJS)], raised liver enzymes [aspartate or alanine transaminase (AST or ALT) >grade 2, i.e. >5× upper limit of normal (ULN)], and acute hepatitis. The characteristics of children developing adverse events were compared with others using Fisher's exact or rank-sum tests. AST and ALT were measured at enrolment and weeks 6, 12, 24, and 24-weekly throughout the study and were matched with nevirapine C_{\min} as described for viral load. The association between nevirapine C_{\min} (and all covariates above and pre-ART more than grade 1 transaminase elevation) and the risk of developing more than grade 1 AST or ALT, that is, more than 2.5× ULN (composite endpoint), was estimated in the ART-naive group as for virological nonsuppression. In addition, in the same group, the change from baseline in transaminase levels at weeks 6, 48, and 96 was compared using Wilcoxon signed rank test separately for children with C_{\min} below and above the threshold identified most predictive of transaminase more than 1 grade elevations by likelihood profiling as explained above. Probabilities of adverse events were similarly estimated using mixed-effects logistic regression.

Results

Patient characteristics

Of 478 children in CHAPAS-3, 338 received nevirapine (99 ART-experienced) combined with a two-NRTI backbone, and contributed 3340 pharmacokinetics samples (1566 dosing intervals, 1–6 per individual) and 718 viral loads after enrolment (1–3 per individual). Sixteen individuals (all ART-naive) changed ART: nine to efavirenz (when TB was diagnosed) and seven to protease-inhibitors [three for adverse events, four for clinical failure (two in year 2 and two in year 3)]. The demographic characteristics and model-estimated pharmacokinetics parameters for children included in this analysis are shown in Table 1 by virological response group.

Amongst ART-naive children, 151 (68%) achieved and maintained viral load less than 100 copies/ml, 125 (56%) by week 48. Those who took longer to suppress had almost three times higher pre-ART viral load and lower CD4⁺ cell counts. Amongst ART-naive participants who suppressed by week 48, 27 rebounded, and the majority of these children resuppressed during follow-up. Pre-ART CD4⁺ cell percentage in these rebounders was lower than other groups, and median pre-ART viral load between that for the suppressed group and for those taking longer to suppress or never suppressed. The remaining 45 ART-naive children (20%) never suppressed viral load to less than 100 copies/ml, but only four showed clinical evidence of treatment failure. Individuals who never suppressed had significantly higher pre-ART viral load than children who suppressed by week 48,

Table 1. Patient characteristics.

Suppression group	Naïve					P**
	Suppressed	Slow suppress	Rebound	Never suppressed	Experienced	
Number of patients (row %)	125 (56%)	26 (12%)	27 (12%)	45 (20%)	99	
Age (years)	2.0 (0.7–4.9)	2.2 (0.9–3.8)	1.9 (1.1–8.5)	1.6 (0.7–3.5)	6.1 (5.1–10.6)	<0.001
Weight (kg)	9.8 (6.4–16.2)	10.4 (6.1–15.4)	9.8 (6.6–19.9)	8.6 (5.8–13.8)	19.0 (15.3–24.5)	<0.001
CD4 ⁺ cell count	21.0 (7.4–42.6)	19.4 (7.6–44.7)	16.5 (6.6–39.4)	19.0 (9.0–38.5)	34.5 (21.9–48.0)	<0.001
percentage (%)						
CD4 ⁺ cell count	1137 (397–3289)	832 (209–1699)	732 (277–2061)	1025 (353–2453)	1259 (627–2237)	<0.001
(cells/ μ l)						
Viral load	245 250	663 047	325 980	555 130	<50	<0.001
(copies/ml)	(6506–1.7 mil)	(149 283–12.7 mil)	(43 144–3.8 mil)	(132 393–5.4 mil)		
Sex (M/F)	68/57	15/11	8/19	28/17	49/50	0.017
pMTCT (Y/N)	22/103	5/21	5/22	6/39	8/91	0.214
WHO Stage						0.532
1	19	4	2	4	22	
2	46	8	10	22	23	
3	53	12	13	14	38	
4	7	2	14	5	16	
Metabolic subgroup	41	11	11	12	32	0.611
EM						
IM	59	8	11	25	41	
SM	24	7	5	7	26	
USM	1	0	0	1	0	
d4T	40	11	11	16	27	0.070
ZDV	36	4	8	17	42	
ABC	49	11	8	12	30	
PK						
C _{min} (mg/l)	5.43 (2.48–14.66)	6.68 (1.79–15.21)	5.21 (1.38–12.33)	4.76 (1.45–9.08)	6.57 (3.44–16.32)	<0.001
AUC (mg/l/h)	80.8 (36.8–196.8)	94.1 (25.2–202.7)	71.9 (17.1–167.6)	66.5 (18.2–128.6)	92.1 (50.1–216.9)	<0.001
C _{max} (mg/l)	7.84 (3.61–17.84)	9.26 (2.66–18.27)	6.87 (1.68–15.39)	6.34 (1.76–12.02)	8.90 (4.96–19.58)	<0.001
CL (l/h)	0.91 (0.44–1.42)	0.99 (0.39–1.41)	0.91 (0.55–1.47)	0.85 (0.52–1.26)	1.20 (0.59–1.90)	<0.001
MEMS score (%)	93.0 (63.1–98.7)	94.9 (76.3–98.9)	92.8 (58.0–97.2)	82.0 (50.0–97.4)	90.2 (59.8–99.2)	0.017
CV C _{min} (%) ^a	31.0 (9.7–237.4)	28.5 (7.4–307.1)	31.0 (10.2–246.6)	45.0 (11.0–395.0)	21.0 (8.0–103.2)	<0.001
CV AUC (%) ^a	32.0 (9.4–291.7)	30.5 (11.5–391.7)	37.0 (8.0–308.0)	65.5 (12.0–420.0)	22.0 (8.9–124.4)	<0.001

Numbers are number or median (5th and 95th percentile). For time-varying PK exposures and adherence, medians are of the geometric mean per child over all follow-up. Presented PK parameters relate to exposures following the morning dose. Included patients received nevirapine and had at least one PK visit. M – male, F – female. pMTCT – exposure to any ART in children or mothers in prevention of mother-to-child transmission (in ART-naïve group 29 children and 28 mothers had any exposure to ART in pMTCT; 20 children were exposed to nevirapine [14 single dose nevirapine (sdNVP)] and six nevirapine > 2 days) and 14 children to zidovudine (five in addition to nevirapine), 25 mothers were exposed to nevirapine (24 sdNVP and one nevirapine 2 days) and five mothers to zidovudine (three in addition to nevirapine in ART-experienced group four children and eight mothers had any exposure to ART in pMTCT; all children were exposed to nevirapine (all sdNVP) and none to zidovudine, all mothers were exposed to nevirapine (all sdNVP) and none to zidovudine. EM (CYP2B6 extensive metabolizers) – 516GGJ983TT; IM (CYP2B6 intermediate metabolizers) – 516GGJ983TC or 516GT1983TT; SM (CYP2B6 slow metabolizers) – 516TT1983TT or 516GT1983TC; USM (CYP2B6 ultraslow metabolizers) – 516GGJ983CC. ABC, abacavir; ART, antiretroviral treatment; AUC, area under the curve; CL, clearance; CV, coefficient of variation; d4T, stavudine; EM, extensive metabolizers; IM, intermediate metabolizers; NRTI, nucleoside reverse transcriptase inhibitor; PK, pharmacokinetics; SM, slow metabolizers; USM, ultraslow metabolizers; ZDV, zidovudine.

^aOnly children with more than one sampling visit.

*Kruskal–Wallis or Fisher's exact test comparing four groups of originally treatment-naïve children only.

**Kruskal–Wallis or Fisher's exact test comparing five groups including children who were treatment-experienced at enrolment.

lower adherence than the other ART-naïve children (82 vs 93%, rank-sum $P=0.005$) and lower nevirapine pharmacokinetics exposures ($P<0.05$) with higher levels of intraindividual variability ($P=0.02$), possibly indicating erratic adherence patterns.

ART-experienced children were much older, with viral load less than 50 copies/ml and higher CD4⁺ cell percentage at enrolment. The average C_{\min} and AUC in this group was also higher than most ART-naïve children ($P<0.001$). Despite comparable MEMS-adherence scores, ART-experienced children had significantly lower intraindividual variability in nevirapine pharmacokinetics measures than ART-naïve children ($P<0.001$), which might suggest more consistent adherence. Virological outcomes remained excellent: 88 (89%) remained suppressed less than 100 copies/ml throughout the study, 10 (10%) had a virological rebound, and only one child had all viral load measurements more than 100 copies/ml.

Concentration–response relationship

Cox repeated failures regression on 437 matched pharmacokinetics viral load measurements in 219 ART-naïve individuals (Table 2) showed that the hazard of nonsuppression decreased by 7% for every 1 mg/l increase in nevirapine C_{\min} (95% CI: 2–12%). The estimated probability of nonsuppression declined from 26% for a nevirapine C_{\min} of 3 mg/l to 18, 12, and 9% for C_{\min} values of 8, 12, and 16 mg/l, respectively, using the mixed-effects repeated measures logistic model (Fig. 1a). Likelihood profiling identified a nevirapine C_{\min} of 10.2 mg/l (95% CI 7.9–11.8) as most predictive of decreased risk of virological nonsuppression (Supplement Fig. S1a, <http://links.lww.com/QAD/B33>). Despite the markedly decreased probability of nonsuppression with

C_{\min} above this threshold and improved specificity and positive predictive value, in comparison with the other C_{\min} cut-offs, the identified threshold had inferior sensitivity, accuracy, and negative predictive power (Table 3).

Predictors of virological nonsuppression

Nevirapine C_{\min} , clinical site, age, WAZ, HAZ, adherence, bCD4⁺ cell percentage, and bVL were all associated with viral load more than 100 copies/ml in univariate analyses ($P<0.2$). However, only C_{\min} , clinical site, adherence, bCD4⁺ cell percentage, and bVL were independent predictors ($P<0.05$). After adjusting for these factors, the effect of C_{\min} dropped slightly from 7 to 6% (95% CI 1–10%) (Table 2). The strongest predictors were adherence and bVL. Every 10% increase in MEMS score was associated with a 22% reduction (95% CI 10–32%), and a 10-fold higher bVL was associated with a 2.08-fold increase (95% CI 1.50–2.90) in the risk of nonsuppression. Furthermore, for every 10% increase in bCD4⁺ cell percentage, the risk of viral nonsuppression was 29% (95% CI 5–46%) lower. The hazard of nonsuppression was significantly greater at two of the three sites in Uganda, even after adjusting for other significant effects (characteristics by site in Supplement Table S1, <http://links.lww.com/QAD/B33>). No significant interactions were detected between predictors in the final model; in particular, there was no evidence that associations between nevirapine exposure and nonsuppression varied by centre (Site 1 – ref, Site 2 – $P=0.23$, Site 3 – 0.51, Site 4 – $P=0.09$).

Adverse events

Skin reactions were rare (four grade-2 HSR, one grade-3 HSR, and one grade-4 SJS). All occurred in ART-naïve

Table 2. Univariate and multivariate predictors of virological suppression on nevirapine.

Factor	Univariate ^a		Final multivariate model ^b	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
C_{\min} (per 1 mg/l higher)	0.93 (0.88–0.98)	0.004	0.94 (0.90–0.99)	0.014
Site (1 ref)	(2) 1.65 (0.91–2.99)	0.096	(2) 1.98 (1.01–3.85)	0.045
	(3) 1.02 (0.54–1.93)	0.943	(3) 1.19 (0.64–2.23)	0.573
	(4) 1.41 (0.72–2.78)	0.315	(4) 2.58 (1.15–5.75)	0.021
Age (per 1 year older)	0.83 (0.71–0.98)	0.034		
Pre-ART CD4 ⁺ cell percentage (per 10% higher)	0.82 (0.65–1.03)	0.101	0.71 (0.54–0.95)	0.019
Pre-ART VL (per 10-fold higher)	2.26 (1.68–3.02)	<0.001	2.08 (1.50–2.90)	<0.001
WAZ (per unit higher)	0.84 (0.69–1.01)	0.069		
HAZ (per unit higher)	0.81 (0.69–0.95)	0.013		
MEMS score (per 10% higher)	0.87 (0.76–0.99)	0.037	0.78 (0.68–0.90)	<0.001
WHO clinical stage (1 ref)	(2) 1.78 (0.81–3.91)	0.154		
	(3) 1.34 (0.61–2.97)	0.466		
	(4) 2.36 (0.87–6.37)	0.090		

ART, antiretroviral treatment; CI, confidence interval; HAZ, height-for-age adjusted Z-scores; VL, viral load; WAZ, weight-for-age adjusted Z-scores.

^aShowing all factors with univariate $P<0.2$, and hence considered for inclusion in the multivariate model.

^bBased on backwards elimination using exit $P>0.05$. HR, hazard ratio, clinical sites: (1) – University Teaching Hospital, Lusaka, Zambia; (2) – Joint Clinical Research Centre, Kampala, Uganda; (3) – Bristol Myers Squibb Children's Clinical Centre of Excellence, Baylor College of Medicine, Kampala, Uganda; (4) – Joint Clinical Research Centre, Gulu, Uganda.

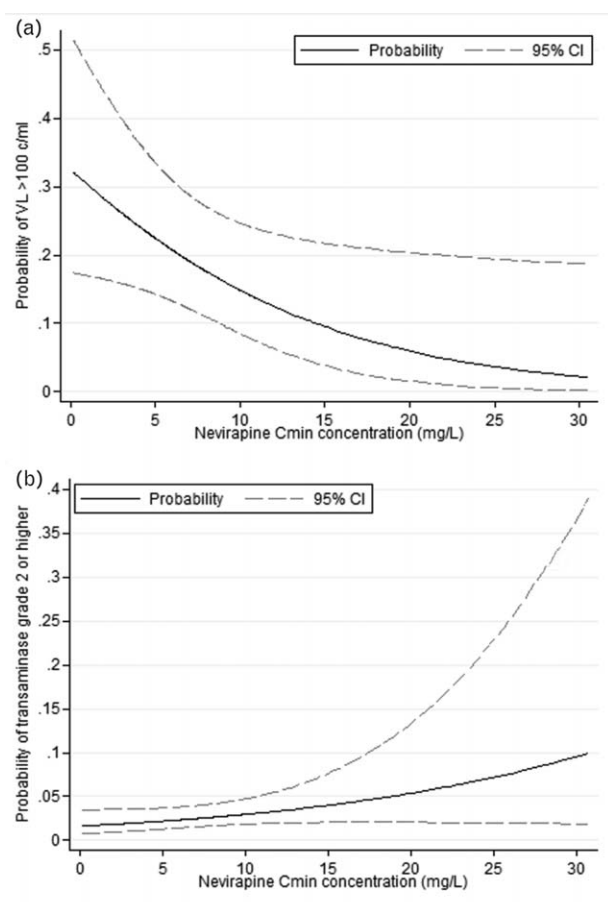


Fig. 1. (a) Probability of nonsuppression (viral load >100 copies/ml) for nevirapine C_{min} , (b) probability of transaminase grade 2 or higher elevations for nevirapine C_{min} .

patients within 2 weeks of ART initiation, and nevirapine was stopped before pharmacokinetics sampling. The mean pre-ART age and $CD4^+$ cell percentage were 2.8 years and 22%, respectively, and did not differ significantly from other children (rank-sum $P > 0.4$); sex was also similar (two boys, four girls; exact $P = 0.43$) as was *CYP2B6*-MET (three extensive metabolizers, two intermediate metabolizers, and one slow metabolizers, exact $P = 0.87$).

Transaminase measurements postbaseline were available for 335 children (2273 samples). At enrolment, AST was significantly higher in ART-naïve than ART-experienced children with median 43 IU (5th–95th: 26–127) vs 32 IU (22–60), P less than 0.001, but ALT was similar with median 21 (9–75) vs 23 (13–53), $P = 0.14$. Transaminase elevations grade 3 and above were rare (15 in total) and were not associated with any particular characteristics (Supplement Table S2, <http://links.lww.com/QAD/B33>), there were no cases of acute hepatitis.

Of 39 more than grade 1 elevations observed in 235 ART-naïve children, 24 (nine both AST and ALT, six AST only,

Table 3. Comparison of previously published treatment targets applied to nevirapine trough concentrations of the current data set, as well as the thresholds derived in this analysis.

Nevirapine target conc. (mg/l)	2.57 (10th percentile) ^a	3.0 ^{7b}	3.5 ^{6b}	4.04 (25th percentile) ^a	4.3 ^{8b}	5.82 (50th percentile) ^a	10.2 ^a
HR (95% CI) ^c	2.05 (1.28–3.30)	1.50 (0.95–2.36)	1.62 (1.08–2.41)	1.62 (1.08–2.41)	1.51 (1.04–2.23)	1.46 (1.02–2.11)	3.05 (1.59–5.86)
P	<T	<T	<T	<T	<T	<T	<T
Samples with VL > 100 copies/ml/VL < 100 copies/ml (n)	19/17	20/28	31/41	40/55	45/68	75/132	126/241
Percentage of samples with VL > 100 copies/ml	52.7%	41.6%	43.0%	42.1%	39.8%	36.2%	34.3%
Sensitivity	14.1%	14.8%	23.0%	29.6%	33.3%	55.6%	93.3%
Specificity	94.4%	90.7%	86.4%	81.8%	77.5%	56.3%	20.2%
Accuracy	69.6%	67.3%	66.8%	65.7%	63.8%	56.1%	42.8%
Positive predictive value	52.8%	41.7%	43.1%	42.1%	39.8%	36.2%	34.3%
Negative predictive value	71.1%	70.4%	71.5%	72.2%	72.2%	73.9%	87.1%

<T – below target, >T – above target. CI, confidence interval; HR, hazard ratio; VL, viral load.

^aCurrent investigation.

^bPreviously published.

^cHazard ratio for concentrations below target compared with above target.

and nine ALT only) were matched with nevirapine concentrations and were included in the Cox repeated failures model. The model identified nevirapine C_{\min} [hazard ratio per unit higher (95% CI) 1.07 (1.01–1.13), $P=0.032$], but no other factors (including baseline transaminase elevation, sex, age, and WAZ/HAZ) to be univariably associated with increased risk of transaminase grade 2 and above elevations. Likelihood profiling identified C_{\min} cut-off of 12.4 mg/l (95% CI 7.7–13.5) with hazard ratio (95% CI) above vs below the identified threshold of 5.18 (1.95–13.80), P less than 0.01, (Supplement Fig. S1b, <http://links.lww.com/QAD/B33>). All the observed transaminase elevations were transient and none led to change in treatment, and although AST and ALT levels were higher in matched samples with nevirapine C_{\min} more than 12.4 mg/l, at most time points the increase from baseline was not statistically significant (Table 4). The probability of transaminase elevations by nevirapine C_{\min} estimated using mixed-effects repeated measures logistic model are presented in Fig. 1b and c and remained below 10% up to 30 mg/l.

Discussion

We observed that virological nonsuppression in a group of African children treated with nevirapine in combination with two NRTIs was affected by nevirapine C_{\min} and treatment adherence, as well as pre-ART viral load and $CD4^+$ cell percentage. Despite confirming a significant concentration–response relationship, we could not identify a meaningful exposure cut-off predictive of virological nonsuppression. Furthermore, other factors independent of systemic exposures were more strongly associated with nonsuppression than nevirapine exposure. Children with lower viral load at ART initiation and better adherence had improved virological outcomes. Adverse events were rare, but high nevirapine C_{\min} was associated with transient grade 1 and above transaminase elevations.

Similar to previous investigations in adults [6,7,29], we confirmed that higher nevirapine concentrations led to superior virological suppression in children. Customarily used efficacy thresholds for nevirapine were derived from distributions of concentrations in adult, predominantly white, patients, even though nevirapine exposures are higher in African populations [10] and children [5,30], bringing into question their universal applicability [4]. We recently proposed an alternative method of selecting an efficacy threshold based on likelihood profiling and successfully used it for efavirenz in a similar population of African children [27]. Interestingly, a similarly clear cut-off could not be derived for nevirapine, in line with findings by Van Leth *et al.* [31]. The identified C_{\min} threshold of 10.2 mg/l, despite having superior sensitivity

Table 4. Transaminase levels at 6, 48, and 96 weeks of treatment, compared with baseline liver enzymes in antiretroviral treatment-naïve children, by nevirapine C_{\min} threshold most predictive of transient grade 2 and above transaminase elevations.

Baseline, N=235	Nevirapine conc. (mg/l)	Week 6, N=196			Week 48, N=200			Week 96, N=188		
		Median ^a	Change from baseline [*]	P^*	Median ^a	Change from baseline [*]	P^*	Median ^a	Change from baseline [*]	P^*
AST (U/l) (25.7–107.9)	<12.4	36.0 (24.2–95.8)	–5.5 (–8.5 to –3.0)	0.001	37.0 (24.0–78.6)	–5.5 (–8.5 to –2.5)	<0.001	35.0 (25.0–71.1)	–7.0 (–10.0 to –4.0)	<0.001
	>12.4	42.0 (24.0–118)	–7.0 (–38.5 to 27.5)	0.407	51.0 (26.8–268.4)	3.8 (–44.0 to 257.0)	0.799	43.0 (31.2–205.0)	8.0 (–34.0 to 116.0)	0.488
ALT (U/l) (9.0–95.8)	<12.4	22.0 (10.0–82.6)	2.5 (0.0–4.5)	0.068	26.5 (13.0–64.6)	5.0 (2.5–8.0)	<0.001	24.0 (8.6–67.2)	1.5 (–1.5–4.0)	0.373
	>12.4	35.0 (9.0–66.5)	9.3 (5.5–16.5)	0.014	37.5 (18.40–215.8)	14.5 (–16.0 to 169.0)	0.441	41.0 (20.2–169.0)	16.0 (–11.0 to 186.5)	0.343

ALT, alanine transaminase; AST, aspartate transaminase.

^aMedian (5th and 95th percentile).

^{*}Median (95% nonparametric confidence interval) of the differences between week 6, 48, and 96 and baseline, respectively, and P value from Wilcoxon signed rank test.

and negative predictive value, had substantially lower specificity and accuracy than other cut-offs (Table 3). In comparison, the threshold identified for efavirenz (C_{\min} of 0.65 mg/l) was visibly superior to previously suggested and clearly predicted nonsuppression, with only 7% of samples above it but 37% below it having viral load more than 100 copies/ml [27]. Nevirapine has lower potency (protein adjusted IC₉₅ of 196.6 vs 54.7 ng/l) [32] and shorter half-life than efavirenz, which is the most potent component of NNRTI + two NRTI ART contributing 65% of its total efficacy [33]. A higher contribution to treatment efficacy of the two accompanying NRTIs may have obscured a clear pharmacokinetics efficacy threshold for nevirapine. The above could also explain why virological outcomes were more strongly related to several other factors than nevirapine exposures in children on nevirapine-based ART.

The effects of pre-ART CD4⁺ cell percentage and viral load on virological outcome have been well documented [34–39]. In CHAPAS-3, ART-naive children on nevirapine with a higher pre-ART viral load either took much longer to achieve viral load less than 100 copies/ml or never suppressed, consistent with the increased hazard of virological nonsuppression with higher pre-ART viral load. The pre-ART CD4⁺ cell percentage in ART-naive children who rebounded after achieving initial suppression by week 48 was significantly lower than in other groups, and it was also an independent predictor of virological nonsuppression. Our findings highlight the benefits of treatment initiation in early stages of disease, in children with a low viral load and high CD4⁺ cell percentage. The recent START trial [40] in adults confirmed the importance of starting ART early, and supported guidelines recommending initiation of ART regardless of CD4⁺ cell count [41].

Our findings emphasize the importance of treatment adherence in achieving and maintaining virological suppression, consistent with other studies in African children [38,39]. Children who never achieved viral load less than 100 copies/ml in our study had significantly lower MEMS scores. Adherence also independently predicted virological nonsuppression with risk decreasing by 22% for every 10% higher MEMS score. It has been hypothesized that adherence above 95% is required to achieve and maintain beneficial effects of ART [31,42]. Interestingly, in CHAPAS-3, the median adherence in children taking efavirenz, an NNRTI administered once a day, was higher than for nevirapine (99 vs 91%) [27]. This could explain why the association between adherence and virological outcome was more significant for nevirapine than efavirenz. Meta-analyses confirm that once-daily regimens and reduced pill burden are associated with higher adherence to ART [43,44]. Lower adherence could be a contributory factor to the higher proportion of ART-naive patients on nevirapine who never achieved viral load less than 100 copies/ml (20 vs

6% on efavirenz) and worse virological outcomes in ART-experienced children. CHAPAS-3 was not designed to compare the effectiveness of nevirapine and efavirenz, but several other studies in children in resource-limited settings suggest better virological outcomes for the latter [36–38,45–47]. Yet, nevirapine is currently the only NNRTI formulated as all-in-one paediatric FDC. Although developing a similar formulation containing efavirenz could improve treatment adherence and hence virological outcome, this is challenging due to the larger efavirenz dose and higher pharmacokinetic variability due to its pharmacogenetics [5,48].

Adverse events were rare in our study, replicating other paediatric investigations [17,18,49]. High nevirapine concentrations were associated with elevated hepatic enzymes in adults, in particular in those with low BMI [12,50], but several other studies including African patients showed a low risk of hepatotoxicity [10,15,16]. In CHAPAS-3, although we detected an association between high nevirapine exposures and increased risk of developing more than grade 1 transaminase elevations, all observed events were transient and did not lead to ART substitutions. Likelihood profiling identified a C_{\min} threshold of 12.4 mg/l as most predictive of these transient events, and although we observed higher transaminase levels during the study when concentrations were above this threshold, they were not significantly different to baseline. Moreover, the baseline values of AST and ALT for ART-experienced children (on nevirapine-based ART for >2 years) were not significantly higher than in ART-naive children. Together, these suggest that these findings may have limited clinical relevance. Recent reports hypothesize that nevirapine-related hepatotoxicity has a genetic cause [51–53]. Similarly, HSRs were rare, possibly due to dose escalation in the first 2 weeks of the study [17]. Small numbers precluded associations with any specific patient characteristics, but they occurred early in the study, before any pharmacokinetics sampling, making it difficult to confirm speculations of their idiosyncratic cause [4,54].

Considering nevirapine's low genetic barrier for viral resistance [8], the risk of nonsuppression decreasing with increasing drug concentrations, and the presented safety profile, maintaining C_{\min} higher than the current target of 3–8 mg/l could have beneficial effects on general treatment outcomes in African children, and nevirapine concentrations as high as 12.4 mg/l should not lead to increased risk of adverse events. Results of recent nevirapine population pharmacokinetic analysis in children from CHAPAS-3 [5] show that currently recommended paediatric dosage [41] provides average C_{\min} at the upper range of the 3–8 mg/l target, even though slow metabolizers determined by *CYP2B6* 516G > T|983T > C genotype are at risk of exposures above 12.4 mg/l [5].

Our study has several limitations. We could not find a plausible explanation for the detected effect of clinical site on virological outcome, which was not due to small imbalances in other factors as these were either adjusted for or had no association with virological nonsuppression. These centre effects likely reflect residual confounding from factors not captured in our study, either differences in other aspects of management on ART or other local variability in the patient populations, for example, in socio-economic status, distance to clinic, and others. However, we found no evidence that the effect of other independent predictors (adherence, viral load, and nevirapine exposure) varied across centres (i.e. no interaction/heterogeneity) supporting generalizability of these findings to other settings. No genotyping was conducted at enrolment, so we were not able to assess the impact of preexisting NNRTI resistance on response. However, we did not find any evidence of an association between pMTCT (predominantly single-dose nevirapine) and increased risk of nonsuppression, similarly to another recent study [39], suggesting that the impact of preexisting NNRTI resistance may be relatively small compared with the other factors assessed. Most viral loads were matched with nevirapine concentrations measured 12 weeks earlier, and one could argue that drug concentrations measured on the same day as viral load could be more predictive of virological outcome. However, suppression is likely related to maintained drug exposure above a certain threshold, and a random measurement in the time period preceding it could be a better indicator of it. Adherence in our study was only measured in certain time periods, and the same drug-taking pattern was assumed to persist until the next measurement. Most children had only three viral loads after enrolment, and our analysis assumed that no viral rebounds occurred in between. Lastly, our findings should not be generalized to ART based on other drugs, in fact, amongst children enrolled to CHAPAS-3, we found different predictors of virological outcome for children on efavirenz.

Conclusion

Higher nevirapine concentrations were associated with significantly better virological outcomes, but a meaningful cut-off predictive of increased risk of non-suppression could not be identified, possibly due to the effects of the combined NRTIs. Lower viral loads at ART initiation and higher treatment adherence were the most predictive determinants of virological suppression. The outcome was further affected by pre-ART CD4⁺ cell percentage and clinical site. Adverse events were rare and, even though we detected an association between nevirapine C_{\min} more than 12.4 mg/l and transaminase elevations, this is of limited clinical relevance due to their transient character. Treatment initiation at lower viral load and higher CD4⁺ cell percentage, increased adherence, and maintaining average C_{\min} higher than current target

could have a positive effect on virological suppression of African children treated with nevirapine.

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Conflicts of interest

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