Effect of Lopinavir and Nevirapine Concentrations on Viral Outcomes in Protease Inhibitor-experienced HIV-infected Children

Retsilisitsoe R. Moholisa, MS, Michael Schomaker, PhD,† Louise Kuhn, PhD,‡ Sandra Castel, PhD,* Lubbe Wiesner, PhD,* Ashraf Coovadia, MD,§ Renate Strehlau, MD,§ Faeezah Patel, MD,§ Francoise Pinillos, MD,§ Elaine J. Abrams, MD,¶ Gary Maartens, MD,*‖ and Helen McIlleron, MD, PhD*‖*

Background: Adequate exposure to antiretroviral drugs is necessary to achieve and sustain viral suppression. However, the target antiretroviral concentrations associated with long-term viral suppression have not been adequately defined in children. We assessed the relationship between plasma lopinavir or nevirapine concentrations and the risk of subsequent viremia in children initially suppressed on antiretroviral therapy.

Methods: After an induction phase of antiretroviral treatment, 195 children with viral suppression (viral load ≤400 copies/mL) were randomized to continue a lopinavir/ritonavir-based regimen or to switch to a nevirapine-based regimen (together with lamivudine and stavudine). Viral load and lopinavir or nevirapine concentrations were measured at clinic visits 4, 8, 12, 16, 20, 24, 36, 52, 64 and 76 weeks post randomization. Cox multiple failure event models were used to estimate the effects of drug concentrations on the hazard of viremia (viral load >50 copies/mL)

Results: At randomization, the median (interquartile range) age, $CD4^+$ T-Lymphocyte percentage, weight-for-age and weight-for-height z scores were 19 (16–24) months, 29% (23–37), −0.6 (−1.3 to 0.2) and −3.2 (−4.1 to −2.1), respectively. The proportion of children with viral load 51–400 copies/mL at randomization was 43%. The hazard of subsequent viremia during follow-up was increased for lopinavir concentrations <1 versus ≥1 mg/L [adjusted hazard ratio 0.62 (95% confidence interval, 0.40–0.94)] and for children with viral loads 51–400 copies/mL at randomization. Nevirapine concentrations were not significantly associated with subsequent viremia.

Conclusions: Plasma lopinavir concentrations predicted viral outcomes in children receiving lopinavir-based antiretroviral therapy. Our findings support a minimum target concentration of ≥1 mg/L of lopinavir to ensure sustained viral suppression.

Key Words: therapeutic drug monitoring, pharmacokinetics, viremia

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Combination antiretroviral therapy (ART) has significantly improved survival and quality of life of HIV-infected children worldwide.[1] The maintenance of adequate drug exposures is necessary to prevent viral resistance and ART failure, and high levels of adherence are critical for maintaining viral suppression.[2,3]

Current ART treatment guidelines for HIV-infected children recommend combination therapy of dual nucleoside analogue reverse transcriptase inhibitor combined with either a non nucleoside reverse transcriptase inhibitor or a boosted protease inhibitor (PI). Nevirapine (NVP) has a low barrier to develop viral resistance. Suboptimal NVP concentrations have been shown to select for the development of drug resistance mutations.[4] An ART regimen including the co-formulated PI lopinavir/ritonavir (LPV/r) has been shown to be superior to a NVP-based regimen for treating infants exposed to NVP perinatally.[5] LPV/r has a high barrier for resistance, however, the oral suspension of LPV/r has poor palatability that may result in poor treatment adherence.[6,7]

Based largely on studies in adults, minimum trough concentrations of 1 and 3 mg/L are recommended for LPV and NVP, respectively.[8,9] Therapeutic drug monitoring (TDM) is recommended by some guidelines for children on LPV or NVP,[10] as the plasma concentrations of both drugs are highly variable even after observed doses. However, few data exist on the relationship between plasma drug concentrations of LPV or NVP, and viral response in children.

We measured serial LPV and NVP concentrations from stored plasma in children enrolled in a clinical trial.[7,10] Previously, we described the increased risk of viremia in children initiated on LPV/r-based ART during the pre randomization phase of the study.[11] Once they had achieved viral suppression (<400 copies/mL), children were randomized to continue LPV/r or to switch to NVP. Here, we use data from the post randomization phase of the trial to evaluate the plasma LPV and NVP concentrations associated with maintenance of viral suppression.

METHODS

Plasma LPV and NVP concentrations were retrospectively analyzed in samples collected from participants of the Neverest2 trial at clinic visits during the post randomization period.[7,12] The Neverest2 trial was a randomized open-label clinical trial investigating treatment options for NVP-exposed children who initiated

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From the *Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa; †Centre for Infectious Diseases Epidemiology and Research, School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa; ‡Gertrude H Sergievsky Center, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY; §Empilweni Services and Research Unit, Rahima Moosa Mother and Child Hospital, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa; ¶ICAP, Mailman School of Public Health, and College of Physicians & Surgeons, Columbia University, New York, NY; and ‖Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa.

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Address for correspondence: Helen McIlleron, PhD, Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa. E-mail: Helen.McIlleron@uct.ac.za.

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PI-based ART when less than 24 months of age. HIV-infected children attending the Rahima Moosa Mother and Child Hospital, Johannesburg, South Africa, who achieved a viral load (VL) ≤400 copies/mL for at least 2 consecutive visits on LPV-based ART were eligible for randomization. Once criteria for randomization were met, the children were randomized 1:1 to continue their LPV/r regimen or switch LPV/r to NVP. NVP (Viramune oral solution, Boehringer Ingelheim) was introduced at 120 mg/m^2 once daily for the first 2 weeks and thereafter at 200 mg/m^2 12 hourly. Children randomized to continue LPV/r (Kaletra oral solution, Abbott Laboratories, Abbott Park, IL) received doses of 230 mg/m^2 12 hourly. Lamivudine and stavudine were used as the other 2 drugs. Doses were adjusted according to the growth of the children at each visit. Both NVP and LPV groups received additional adherence counseling, including specific instructions concerning the lead-in schedule and possible adverse effects for children switching to NVP.

Data collected at randomization included age, sex, VL and CD4$^+$ T-lymphocyte percentage (CD4%). Weight-for-age z score (WAZ) and height-for-age z score (HAZ) at randomization were calculated using WHO software.[7] In both groups, blood samples were collected at randomization and at 4, 8, 12, 16, 20, 24, 36, 52, 64 and 76 weeks post randomization and at unscheduled clinic visits. Blood samples collected at each visit post randomization were used to measure VL (post randomization viremia was defined as VL >50 copies/mL) and LPV or NVP concentrations. The time of blood sample collection was documented, as was the time of the morning dose of antiretrovirals, as reported by the caregiver. Caregivers were requested to return medication bottles at each visit. The bottles were weighed and the contents reconciled with the expected usage of each medication to determine the degree of adherence. Adherence was defined as returning less than 20% of the expected volume of any of the 3 drugs, whereas returning more than 20% was defined as non adherence. In children who were diagnosed with tuberculosis (TB) after randomization, concomitant TB treatment was recorded at each visit. After 76 weeks, all children were enrolled in an extended follow-up period during which clinical care was provided and monitored.

Laboratory Methods

Plasma LPV and NVP concentrations were assayed using validated liquid chromatography tandem mass spectrometry methods developed in the Division of Clinical Pharmacology, Cape Town, South Africa. An AB Sciex 4000 mass spectrometer was operated at unit resolution in the multiple reaction monitoring mode. The validated concentration range for the LPV assay was 0.16 to 20 mg/L and that for NVP was 0.1 to 15 mg/L. Inter- and intraday coefficients of variation were below 10% for all quality control concentrations. The laboratory, at which the concentrations were assayed, participates in the International Inter-laboratory Pharmacology Quality Control Program, the AIDS Clinical Trial Group.

Statistical Analysis

Children with a WAZ > −2 standard deviation below the norm were categorized as underweight. HAZ < −2 was regarded as indicating stunting. Immunity at randomization was categorized as low (CD4% <25%) or high (CD4% ≥25%), whereas VL was categorized as low-level viremia (VL 51–400 copies/mL) or suppressed (VL ≤50 copies/mL). TB treatment was a dichotomous variable (present or absent at each post randomization visit). LPV and NVP concentrations below the limit of quantification (BLQ) were assigned values of 0.08 and 0.05 mg/L, respectively (half the limit of quantification). Characteristics at randomization were described with summary statistics [median, interquartile range (IQR) and proportions].

Cox proportional hazard regression for multiple failure events was used to estimate the crude and adjusted hazard ratios (HRs) for viremia (VL >50 copies/mL) associated with the following pre determined variables: CD4%, age, WAZ, HAZ and VL at randomization, TB treatment post randomization and LPV or NVP concentration at the current visit. In secondary analyses, we determined the crude and adjusted hazards of viremia associated with LPV or NVP concentration at the previous visit, and the crude and adjusted hazards of viremia associated with the average of 2 drug concentrations, derived from the current and prior visits, respectively. To account for missing CD4% and adherence data, as well as LPV and NVP concentrations, 10 multiple imputations were conducted using the Amelia II software package in R.[13] The imputation model included variables for WAZ, HAZ, VL and CD4% at the time of randomization, as well as repeated measures of adherence, TB treatment and NVP and LPV concentrations during follow-up, along with the time (weeks on treatment). All results in our crude and adjusted analyses are based on the imputed datasets and combined using Rubin's rules.[14] HRs are reported together with the 95% confidence intervals (CIs). Akaike information criterion (AIC) for each imputed dataset was used to compare all the adjusted models.

We modeled the effect of LPV and NVP on the hazard not only linearly but also using binary cutoffs for drug concentrations. We used predictive modeling via additive logistic regression models to describe the hazard of viremia (VL >50 copies/mL) for concentrations below each cutoff value, respectively, compared with higher concentrations. Multivariate models were used to adjust for the time post randomization (in weeks), and clustering by individual was used. We compared LPV cutoffs (0.5, 1, 2, 3, 4, 5 and 6 mg/L) and NVP cutoffs (2, 3, 4, 5, 6, 7 and 9 mg/L) by means of generalized cross validation (GCV).[15] We used the model which minimized the GCV criterion, because this minimizes the expected prediction error.

To graphically display the nonlinear effects of LPV and NVP concentrations on the hazard of viremia, we used penalized splines.[16]

Finally, we compared 2 adjusted models by means of AIC in each imputed dataset; the first model included all variables at randomization and LPV or NVP concentrations at each visit, whereas the second model included all variables at randomization and percentage adherence at each visit in both the LPV and the NVP groups. Data were analyzed using the statistical software package R.[17]

RESULTS

Study Population

A total of 195 children from the initial 322 children were enrolled in the post randomization phase of the NEVEREST2 study. Of the 195 children, 96 were switched to NVP, whereas 99 remained on a LPV regimen. Table 1 shows the characteristics of children in both groups in the study at randomization, and indicates missing data. The characteristics in the 2 groups were similar.

Plasma LPV and NVP Concentrations

For the LPV group, a total of 1134 plasma samples from 99 children with a median of 8 samples per child were collected from 3 weeks to 209 weeks post randomization. The blood was sampled a median 3.00 (IQR 2.00–3.91) hours after the reported dose of antiretrovirals, and 7% of the samples were BLQ with 6% missing. The median population LPV concentrations were determined at 24, 50, 76, 100 and 150 weeks, respectively, and were similar across all visits (Table 2).

For the NVP group, a total of 764 samples plasma samples from 96 children, with a median of 6 samples per child, were collected from 3 to 196 weeks post randomization. For the NVP group, a total

TABLE 1. Characteristics at Randomization of the 195 HIV-infected Children Remaining on a Lopinavir/Ritonavir-based Regimen or Switched to Nevirapine-based Antiretroviral Therapy

	Characteristics of Children in the LPV Group (n = 99)			Characteristics of Children in the NVP Group (n = 96)		
Variable	Median	IQR	Missing Data	Median	IQR	Missing Data
Age (mo)	20	16–25	0	18	15–22.25	0
VL ≤50 copies/mL	55 (56%)			53 (55%)		
VL51-400 copies/mL	44 (44%)		0	43 (45%)		0
CD4%	28.05	21.65–35.20	4	29.55	22.95–36.70	3
WAZ	−0.60	−1.26 to 0.07	0	−0.60	−1.17 to −0.13	0
HAZ	−3.18	−3.97 to −1.97	0	−2.80	−2.10 to −4.05	0
TB treatment						0
No	86 (86%)		0	91 (94%)		
Yes	13 (14%)			6 (6%)		

Data are in median (IQR) or n (%).

Age indicates age at randomization; CD4%, $CD4^+$ T-lymphocyte percentage at randomization; HAZ, height-for-age *z* score at randomization; TB treatment, concomitant tuberculosis treatment post randomization; VL, viral load at randomization; WAZ, weight-for-age z scores at randomization.

of 764 samples plasma samples from 96 children, with a median of 6 samples per child, were collected from 3 to 196 weeks post randomization. The median time of sampling was 3.00 (IQR 2.17–3.92) hours after the reported dose, and 1% of samples were BLQ and 9% were missing. As with the LPV concentrations, the median NVP concentrations were similar across all visits (Table 2). Five children had exceptionally high NVP concentrations, that is, NVP concentrations consistently above 40 mg/L for an average of 2 visits. The data for these 5 children were excluded in subsequent analyses. Two of these children developed TB post randomization and were switched to another ART regimen in line with standard practice. One child who experienced toxicity and 1 child with viral failure were withdrawn, and 1 child lost to follow-up.

Predictors of Viremia (VL >50 Copies/mL) in the LVP Group

As shown in Table 3, the risk of viremia (VL >50 copies/mL) was estimated to be reduced by 5% for each 1 mg/L increment in the current visit LPV concentration [HR: 0.95 (95% CI: 0.92, 0.98); $P < 0.01$). Children with low-level viremia (VL 51–400 copies/mL) at the time of randomization had a 2.62-fold increased risk of viremia [HR: 2.62 (95% CI: 1.62, 4.24); $P < 0.01$; Table 3] compared with children with VL <50 copies/mL. After adjusting for other covariates, both LPV concentrations [HR: 0.96 (95% CI: 0.94, 0.99); $P = 0.01$] and VL at randomization [HR: 2.66 (95% CI: 1.68, 4.22); $P < 0.01$] remained significant predictors of post randomization viremia. We found that the average of 2 LPV concentrations (at the current visit and previous visit, respectively) were predictive of viremia in the crude [HR: 0.94 (95% CI: 0.91, 0.98); $P < 0.01$] and adjusted [HR: 0.96 (95% CI: 0.92, 1.00); $P = 0.05$] models (data not shown), whereas LVP concentrations at the previous visit was less predictive of viremia in both crude [HR: 0.98 (95% CI: 0.96, 1.01); $P = 0.15$] and adjusted [HR: 0.99 (95% CI: 0.96, 1.02); $P = 0.36$] models (data not shown). The effect of low-level viremia at randomization remained significant in all models. When we compared the 3 models by means of AIC in each imputed dataset, we showed that the models which included current visit LPV concentrations or the average of LPV concentrations at 2 visits described the data better than the model with previous visit LPV concentrations. Due to high percentage of missing data (24%), adherence was not included in the primary analysis. However, in a secondary analysis, we compared 2 models using AIC in each imputed dataset, where the first model included current LPV concentrations and the second model included recorded adherence (data not shown). In each imputed dataset, the model including LPV (low AIC values) was more predictive of viremia compared with model with adherence.

We used predictive modeling to compare logistic regression models (using GCV values) and thereby evaluating the effects of various cutoff concentrations, we showed that a cutoff concentration of 1 mg/L best predicted viremia (Fig. 1). A separate Cox regression model, in which LPV concentrations were dichotomized with a cutoff of 1 mg/L (Table 3), a 41% reduction in the risk of viremia in children with LPV concentrations ≥1 mg/L compared with children with LPV concentrations <1 mg/L was shown. These associations were preserved in the adjusted models in which low-level viremia at randomization was also significantly associated with increased hazard of viremia.

Predictors of Viremia (VL >50 Copies/mL) in the NVP Group

We assessed the risk of viremia (VL >50 copies/mL) in the NVP group using Cox proportional hazards models. Current visit concentrations (Table 4), previous visit (data not shown) NVP concentrations or average of 2 NVP (data not shown) concentrations, taken at the current and previous visits respectively, were associated with the risk of viremia in crude or adjusted models. We compared the 3 models by means of AIC in each imputed dataset and showed that the model with current visit and average of 2 visit NVP concentrations described the data similarly but better compared with the model with previous visit NVP concentration. Consistently high NVP concentrations were measured in 5 children; these outlying observations were excluded in the primary analysis. However, in the sensitivity analysis, we included the 5 children and they were influential, biasing results to significance (data not shown). Due to high percentage of missing data (29%), adherence was not included in the primary analysis. As for the LPV arm, we showed that current visit NVP concentrations described the data better than recorded adherence in a sensitivity analysis (data not shown).

Based on GCV values for the logistic regression evaluating the effect of NVP concentration thresholds, an NVP concentration cutoff values of 5 mg/L best predicted viremia in the respective arm (Fig. 1). A separate Cox regression model was performed where NVP was dichotomized to evaluate the effects of NVP concentrations ≥5 versus <5 mg/L. While not statistically significant, there was a trend to a reduction in the risk of viremia in children with NVP concentrations ≥5 mg/L [crude HR: 0.64 (95% CI: 0.33, 1.27); $P = 0.20$; Table 4).

TABLE 2. Lopinavir and Nevirpine Concentrations, Time of Sampling, Body Weight and Dose by Study Week

Characteristics	LPV Group						NVP Group					
Weeks	24 (n = 99)	50 (n = 94)	76 (n = 99)	100 (n = 98)	124 (n = 78)	150+ (n = 64)	24 (n = 92)	50 (n = 75)	76 (n = 71)	100 (n = 61)	124 (n = 44)	150+ (n = 34)
Median [Drug] (mg/L)	8.92 (5.34–12.9)	9.19 (5.51–13.9)	10.20 (6.68–13.9)	9.69 (6.28–13.9)	11.60 (7.90–17.2)	11.90 (8.81–14.7)	9.1 (8.4–15.2)	9.51 (7.2–11.4)	11.2 (8.3–14.2)	11.85 (9.2–16.1)	12.4 (9.4–14.7)	11.85 (8.04–16)
% samples between LLQ and 1 mg/L for LPV, between LLQ and 3 mg/L for NVP	8	12	10	9	9	7	3	6	2	4	3	7
% of all samples below 1 or 3 mg/L that were BLQ for LPV/NVP, respectively	75	100	90	55	100	85	0	33	50	75	33	14
Time after dose (h)	3 (2.50–4.25)	3.00 (2.18–4.00)	3.08 (2.33–4.00)	3.33 (2.25–4.25	3.00 (2.25–3.75)	2.83 (2.00–3.67)	3.08 (2.25–3.92)	3.17 (2.58–4.17)	3.33 (2.50–4.17)	3.08 (2.25–4.17)	3.25 (2.58–3.88)	3.00 (2.33–4.00)
Total body weight (kg)	11 (10–12)	12 (11–13)	13 (12–14)	13 (12–16)	16 (15–18)	17 (16–19)	11 (10–13)	12 (10–13)	13 12–14)	14 (12–15)	15 (13–16)	17 (15–18)
Dose (mg/m^2)	228 (222–232)	225 (219–231)	226 (221–231)	226 (223–232)	227 (223–231)	227 (223–231)	196 (193–200)	196 (192–200)	195 (192–198)	197 (193–199)	197 (192–199)	197 (195–200)

Data are in median (IQR), unless otherwise stated.
BLQ indicates below the assay limit of quantification; LLQ, lower limit of quantification; LPV, lopinavir; NVP, nevirapine.

TABLE 3. Cox Proportional Hazards Regression Analysis Describing the Risk of Viremia (VL >50 Copies/mL) Post Randomization in 99 Children Randomized to LPV/r-based Treatment

	Crude		Adjusted	
Characteristic	HR (95% CI)	*P*	HR (95% CI)	*P*
LPV (mg/L)	0.95 (0.92–0.98)	<0.01	0.96 (0.94–99)	0.01
Age ≥20 months	Reference		Reference	
Age <20 months	1.48 (0.91–2.39)	0.11	1.47 (0.92–2.34)	0.11
Normal WAZ	Reference		Reference	
Underweight	2.36 (0.77–7.25)	0.13	2.62 (0.94–7.24)	0.06
Normal HAZ	Reference		Reference	
Stunted	0.64 (0.36–1.12)	0.12	0.78 (0.43–1.39)	0.40
VL ≤50	Reference		Reference	
VL 51–400	2.62 (1.62–4.24)	<0.01	2.66 (1.68–4.22)	<0.01
CD4% ≥25	Reference		Reference	
CD4% <25	1.25 (0.75–2.06)	0.38	1.05 (0.66–1.66)	0.85
TB treatment (no)	Reference		Reference	
TB treatment (yes)	0.36 (0.13–1.05)	0.07	0.41 (0.15–1.15)	0.09
LPV <1 mg/L	Reference		Reference	
LPV ≥1 mg/L)	0.59 (0.40–0.94)	0.03	0.62 (0.40–0.95)	0.03
Age ≥20 months	Reference		Reference	
Age <20 months	1.48 (0.91–2.39)	0.34	1.47 (0.92–2.34)	0.09
Normal WAZ	Reference		Reference	
Underweight	2.36 (0.77–7.25)	0.13	2.90 (1.04–8.42)	0.05
Normal HAZ	Reference		Reference	
Stunted	0.64 (0.36–1.12)	0.16	0.76 (0.43–1.34)	0.34
VL ≤50	Reference		Reference	
VL 51–400	2.62 (1.62–4.24)	<0.01	2.73 (1.72–4.33)	<0.01
CD4% ≥25	Reference		Reference	
CD4% <25	1.25 (0.75–2.06)	0.28	1.05 (0.66–1.66)	0.85
TB treatment (no)	Reference		Reference	
TB treatment (yes)	0.36 (0.13–1.05)	0.07	0.41 (0.15–1.15)	0.09

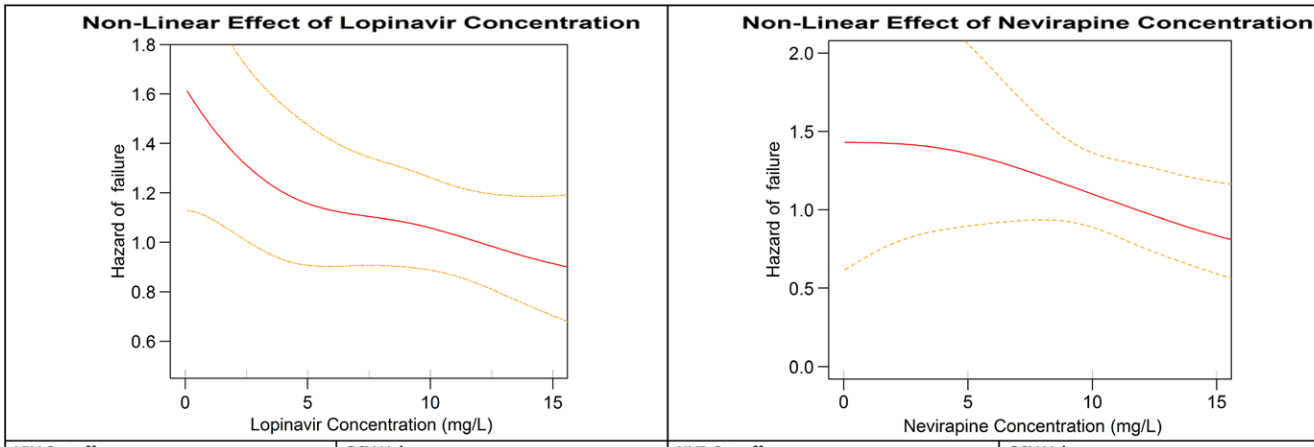
The models described in the first 13 rows describe risk of viremia for each 1 mg/L increment in the current visit LPV concentration, whereas those below that present the effect of the generalized cross validation-determined cutoff of lopinavir.

Age indicates age at randomization; CD4%, CD4+ T-lymphocyte percentage at randomization; HAZ, height-for-age z score at randomization; LPV, lopinavir concentration at each visit; TB treatment, concomitant tuberculosis treatment initiated post randomization; VL, viral load at randomization; WAZ, weight-for-age z scores at randomization.

DISCUSSION

We evaluated the risk of viremia (VL >50 copies/mL) in treatment-experienced children achieving viral suppression (VL <400 copies/mL) after switching to NVP or remaining on LVP/r. In keeping with our analysis of the pre randomization phase of the same study,[11] higher LPV concentrations are associated with sustained viral suppression. Our data suggest that children with LPV concentrations ≥1 mg/L have a reduction in the risk of viremia of about 40%, compared with children with LPV <1 mg/L. In children established on ART, a LPV concentration of 1 mg/L (taken 2–4 hours after the claimed morning dose time) may therefore be used as a threshold for TDM.

We compared different concentrations cutoffs for LPV and NVP, and found 1 and 5 mg/L, respectively, to be the most predictive threshold values for the risk of viremia. However, the association between NVP concentration <5 mg/L and viremia was not significant in the regression model. This finding is consistent with other reports that NVP concentrations do not predict viral response.[9] We acknowledge that there was some model selection uncertainty in the choice of the best cutoff. We used cross validation to find a model that minimizes the expected prediction error; however, it may well be that models with other cutoffs have a good predictive ability too.[18,19] Pre existing drug resistance most likely accounts for the viremia in the NVP group as all children enrolled in this trial had past exposure to single-dose NVP used for PMTCT.[7,11]



LPV Cut-off	GCV Value	NVP Cut-off	GCV Value
0.5 mg/L	0.8363767	2 mg/L	0.6384146
1 mg/L	0.8362492	3 mg/L	0.6384466
2 mg/L	0.8382971	4 mg/L	0.6396787
3 mg/L	0.8376631	5 mg/L	0.6355073
4 mg/L	0.8407137	6 mg/L	0.6374518
5 mg/L	0.8393880	7 mg/L	0.6377969
6 mg/L	0.8448085	9 mg/L	0.6385802

FIGURE 1. Nonlinear effect of lopinavir and nevirapine concentrations on the hazard of viremia with determination of cutoffs using generalized cross validation. Left panel demonstrates lopinavir and the right panel presents nevirapine. GCV indicates generalized cross validation values; LPV cutoff, lopinavir concentrations cutoffs; NVP cutoff, nevirapine concentrations cutoffs.

Low-level viremia at randomization was associated with increased risk of ongoing viremia. This finding was more marked in children on the LPV/r-based regimen, and was independent of other effects captured by the multivariate models. This suggests that the risk of future viremia, conferred by low-level viremia at randomization, was not modified by LPV exposure post randomization; however, our study was not designed to evaluate whether interventions to increase LPV exposure in those children with low-level viremia would lead to suppression.

In contrast to NVP, a high proportion (55%–100%) of LPV concentrations below the threshold of 1 mg/L were below the quantifiable limit of the LPV assay (0.16 mg/L) across all visits (Table 2). This suggests that poor adherence accounts for most LPV concentrations <1 mg/L, which were associated with viremia, and supports efforts to develop LPV/r formulations with improved palatability. Sensitivity analyses, in which LPV concentrations below the quantifiable limit were excluded, support the notion that the very low LPV concentrations drive the association with viremia (data not shown). Those poor outcomes were related to drug exposure in 3 of 5 children with exceptionally high NVP concentrations cannot be excluded, and whether the high concentrations were related to genetic polymorphisms of genes regulating the disposition of NVP is the subject of further investigation.

This study has several limitations that are worth highlighting. First, antiretroviral drug dosing was not directly observed therefore, our only measure of adherence was caregiver-reported adherence, which likely contributed to intraindividual variability in LPV and NVP concentrations. Second, the time of sampling in relation to the dose is a key determinant of drug concentration. In this study, we did not observe the time of dosing and this was not included in our analysis. Despite this limitation, we have shown that LPV concentrations taken after 3.0 (2.0–3.9) hours after the last dose predict viremia suggest that a sample taken at a routine morning clinic can be used for LPV concentration monitoring. Third, there were missing data, which was dealt by multiple imputations. Previous studies have shown multiple imputations to be superior to complete case analysis in which only patients with complete data across all variables are analyzed.[20] If data are missing at random and thus the probability for value to be missing randomly depends only on observed quantities, then no bias is introduced. We assumed data to be missing at random and found it to be a reasonable assumption given that the missing data related mainly to insufficient sample volumes or lost samples. Finally, we acknowledge that there was some model uncertainty in the choice of the best cutoff. Nonetheless, we used GCV to find a model that minimizes the expected prediction error, however, it may well be that other models with other cutoffs have good predictive ability too.

Strengths of the study include a relatively large sample size with VL and plasma drug concentration measurements at repeated clinic visits, which made it possible to assess the relationship between each child's LPV or NVP concentration and their VL at successive intervals.

Measuring drug concentrations can be used as an effective tool in ensuring that therapeutic targets of ART are met.[21,22] However, TDM is not routinely used in any low and middle income country programs to our knowledge. There is also currently insufficient knowledge of target plasma concentrations in children. Moreover, although minimum trough concentrations for LPV and NVP of 1 mg/L (in treatment naïve children) and 3 mg/L, respectively, have been recommended,[7,20] it is challenging to obtain a sample 12 hours post dose in clinical practice. Our findings suggest that a single sample taken 2–4 hours after the dose is a useful predictor of viremia, at least for LPV, and can be used for TDM.

TABLE 4. Cox Proportional Hazards Regression Model Describing the Risk of Viremia (VL >50 Copies/mL) in Children (n = 96) Associated With Current Visit Plasma Nevirapine Concentrations

	Crude		Adjusted	
Characteristic	HR (95% CI)	*P*	HR (95% CI)	*P*
NVP (mg/L)	0.95 (0.90–1.01)	0.11	0.96 (0.91–1.01)	0.13
Age ≥18 months	Reference		Reference	
Age <18 months	1.31 (0.69–2.47)	0.42	1.48 (0.77–2.84)	0.26
Normal WAZ	Reference		Reference	
Underweight	1.28 (0.37–4.44)	0.69	1.39 (0.34–5.62)	0.64
Normal HAZ	Reference		Reference	
Stunted	0.87 (0.46–1.66)	0.67	0.88 (0.47–1.65)	0.67
VL ≤50	Reference		Reference	
VL >50	1.69 (0.91–3.16)	0. 09	1.75 (0.92–3.35)	0.09
CD4% ≥25	Reference		Reference	
CD4% <25	1.21 (0.61–2.49)	0.59	1.23 (0.63–2.39)	0.64
TB treatment (no)	Reference		Reference	
TB treatment (yes)	1.12 (0.34–3.64)	0.86	1.19 (0.28–5.33)	0.82
NVP ≥5 mg/L	Reference		Reference	
NVP <5 mg/L	0.64 (0.33–1.27)	0.20	0.69 (0.33–1.41)	0.31
Age ≥18 months	Reference		Reference	
Age <18 months	1.31 (0.69–2.47)	0.42	1.48 (0.77–2.82)	0.24
WAZ (normal)	Reference		Reference	
WAZ (advanced)	1.28 (0.37–4.44)	0.69	1.43 (0.36–5.64)	0.61
HAZ (normal)	Reference		Reference	
HAZ (advanced)	0.87 (0.46–1.66)	0.67	0.90 (0.47–1.73)	0.76
VL ≤50	Reference		Reference	
VL >50	1.69 (0.91–3.16)	0. 09	1.79 (0.93–3.44)	0.08
CD4% ≥25	Reference		Reference	
CD4% <25	1.21 (0.61–2.49)	0.59	1.19 (0.61–2.31)	0.61
TB treatment (no)	Reference		Reference	
TB treatment (yes)	1.12 (0.34–3.64)	0.86	1.15 (0.26–5.11)	0.85

The first 13 rows presents the risk of viremia for each 1 mg/L increment of nevirapine concentrations at the current visit, whereas rows below that present the risk of viremia associated with nevirapine concentrations below cutoff value determined using generalized cross validation.

Age indicates age at randomization; CD4%, CD4+ T-lymphocyte percentage at randomization; HAZ, height-for-age z score at randomization; NVP, nevirapine concentration at each visit; TB treatment, concomitant tuberculosis treatment post randomization; VL, viral load at randomization; WAZ, weight-for-age z scores at randomization.

In conclusion, LPV concentrations were associated with the hazard of viremia. Our analysis suggests that LPV plasma concentration monitoring at a routine clinic visit may be a useful tool in identifying sub therapeutic antiretroviral concentrations in children, and thereby assist with adherence support.

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