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JAIDS-JOURNAL OF ACQUIRED IMMUNE DEFICIENCY SYNDROMES, PHILADELPHIA, v. 60, n. 2, pp. 214-218, JUN 01, 2012

Evaluation of Viral Load Thresholds for Predicting New World Health Organization Stage 3 and 4 Events in HIV-Infected Children Receiving Highly Active Antiretroviral Therapy

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Background: This study evaluated a wide range of viral load (VL) thresholds to identify a cut-point that best predicts new clinical events in children on stable highly active antiretroviral therapy (HAART).

Methods: Cox proportional hazards modeling was used to assess the adjusted risk for World Health Organization stage 3 or 4 clinical events (WHO events) as a function of time-varying CD4, VL, and hemoglobin values in a cohort study of Latin American children on HAART ≥6 months. Models were fit using different VL cut-points between 400 and 50,000 copies per milliliter, with model fit evaluated on the basis of the minimum Akaike information criterion value, a standard model fit statistic.

Results: Models were based on 67 subjects with WHO events out of 550 subjects on study. The VL cut-points of >2600 and >32,000 copies per milliliter corresponded to the lowest Akaike information criterion values and were associated with the highest hazard ratios (2.0, $P = 0.015$; and 2.1, $P = 0.0058$, respectively) for WHO events.

Conclusions: In HIV-infected Latin American children on stable HAART, 2 distinct VL thresholds (>2600 and >32,000 copies/mL) were identified for predicting children at significantly increased risk for HIV-related clinical illness, after accounting for CD4 level, hemoglobin level, and other significant factors.

Key Words: pediatric HIV infection, viral load monitoring, viral load threshold, Latin America

(*J Acquir Immune Defic Syndr* 2012;60:214–218)

INTRODUCTION

The importance of quantitative HIV RNA, or viral load (VL), assays for monitoring the treatment of HIV infection is well established for adults and children and is considered standard practice in higher-resource settings.[1,2] VL before initiation of highly active antiretroviral therapy (HAART) has been established as an independent predictor of disease progression in children.[3–5] We previously demonstrated the value of VL as an independent predictor of new HIV-related World Health Organization stage 3 and 4 events (WHO events) in a cohort of Latin American children on HAART.[6] In that study, most recent VL above the 5000 copies per milliliter level used in the WHO definition for virological failure[7] was predictive of WHO events, independent of CD4-defined immunosuppression, hemoglobin level, and other factors; in contrast, the commonly used VL threshold of ≥400 copies per milliliter was not an independent predictor of WHO events in that study The present analysis used the same data set to explore VL thresholds between 400 and 50,000 copies per milliliter to identify the cut-point for the best-fitting model for predicting new WHO events in children on stable HAART (≥6 months of continuous HAART).

METHODS

Details of the NISDI (*Eunice Kennedy Shriver* National Institute of Child Health and Human Development International Site Development Initiative) pediatric protocol, eligibility criteria for this analysis, and methods used when applying Cox proportional hazards modeling to assess the time to WHO events as a function of time-varying CD4-defined immunologic

Received for publication October 26, 2011; accepted January 30, 2012.

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Supported by National Institute of Child Health and Human Development Contracts N01-HD-3-3345 (2002–2007) and HHSN267200800001C (NICHD Control: N01-HD-8-0001) (2007–2012).

The authors have no funding or conflicts of interest to disclose.

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TABLE 1. Summary of Results for Fitting Proportional Hazards Regression Models for Predicting the Occurrence of WHO Events Using Different Cut-Points for VL

		VL		*P*	
VL Cut-Point	**AIC**	**HR (95% CI)**	***P***	**CD4**	**Hemoglobin**
400	731.148	1.61 (0.88 to 2.96)	0.12	<0.001	0.001
1000	730.969	1.61 (0.90 to 2.87)	0.11	<0.001	0.001
1500	730.547	1.66 (0.94 to 2.93)	0.08	<0.001	0.002
2000	729.547	1.78 (1.01 to 3.15)	0.047	<0.001	0.002
2100	729.249	1.82 (1.03 to 3.22)	0.040	<0.001	0.002
2200	728.686	1.88 (1.07 to 3.33)	0.030	<0.001	0.002
2300	728.501	1.91 (1.08 to 3.37)	0.027	<0.001	0.002
2400	728.218	1.94 (1.10 to 3.42)	0.023	<0.001	0.002
2500	727.842	1.98 (1.12 to 3.50)	0.019	<0.001	0.002
2600	**727.426**	**2.02 (1.15 to 3.58)**	**0.015**	<0.001	**0.002**
2700	728.472	1.89 (1.08 to 3.32)	0.026	<0.001	0.002
2800	728.214	1.92 (1.10 to 3.37)	0.023	<0.001	0.002
2900	729.199	1.80 (1.03 to 3.14)	0.038	<0.001	0.002
3000	729.073	1.82 (1.04 to 3.17)	0.036	<0.001	0.002
3500	730.179	1.67 (0.97 to 2.88)	0.07	<0.001	0.001
4000	730.218	1.66 (0.97 to 2.86)	0.07	<0.001	0.002
4500	729.704	1.73 (1.00 to 2.97)	0.049	<0.001	0.002
5000	729.003	1.81 (1.05 to 3.11)	0.033	0.001	0.002
7000	730.003	1.69 (0.99 to 2.88)	0.057	<0.001	0.002
9000	730.151	1.67 (0.98 to 2.86)	0.0608	<0.001	0.002
10,000	732.986	1.25 (0.74 to 2.13)	0.41	<0.001	<0.001
15,000	732.025	1.41 (0.84 to 2.39)	0.20	<0.001	<0.001
20,000	730.618	1.61 (0.94 to 2.73)	0.0778	<0.001	<0.001
25,000	729.574	1.75 (1.03 to 2.99)	0.0402	0.001	<0.001
30,000	727.356	2.02 (1.18 to 3.45)	0.0105	0.002	0.001
31,000	726.737	2.09 (1.22 to 3.58)	0.0073	0.003	0.001
32,000	**726.345**	**2.13 (1.25 to 3.66)**	**0.0058**	**0.003**	**0.001**
33,000	727.481	2.02 (1.17 to 3.47)	0.0112	0.002	0.001
34,000	728.294	1.93 (1.12 to 3.32)	0.0180	0.002	0.001
35,000	727.501	2.03 (1.18 to 3.49)	0.0111	0.002	0.001
40,000	729.282	1.84 (1.06 to 3.22)	0.0318	0.001	<0.001
45,000	730.121	1.75 (0.99 to 3.10)	0.0533	0.001	<0.001
50,000	731.102	1.64 (0.09 to 2.94)	0.1005	<0.001	<0.001

Models having the minimum AIC values among competing models represent the best fit to the data (indicated in bold).
WHO, World Health Organization; VL, viral load; CI, confidence interval; AIC, Akaike information criterion; HR, hazard ratio; CD4, CD4+ T-lymphocyte.

status, VL, and hemoglobin level and time-fixed covariates have been published.[6]

Statistical Analyses

The primary outcome measure was the first occurrence of a new WHO stage 3 or 4 event after the time of eligibility for this analysis. The analyses were based on fitting a proportional hazards regression model to the data repeatedly, each time using a different cut-point for the HIV-1 RNA (VL), with VL, CD4 immunosuppression, and hemoglobin level treated as time-varying predictors. Additional covariates found to be independently associated with the occurrence of WHO events in the prior publication were also included in the modeling. The cut-point for the initial series of models was set at 500 copy intervals for VL up to 5000 copies per milliliter and at 5000 copy intervals for VL from 5000 to 50,000 copies per milliliter. A second series of models was fit to the data with cut-points varying by only 100 copies per milliliter, from 2000 to 3000 copies per milliliter, and by 1000 copies per milliliter from 30,000 to 35,000 copies per milliliter, when honing in on the best-fitting model for predicting WHO events. Model selection was based on the Akaike information criterion (AIC), for which the model having the minimum AIC value among competing models represents the best fit to the data.[8] All analyses were conducted using the SAS statistical software, version 9.0 (SAS Institute Inc, Cary, NC).

RESULTS

The models were based on 550 subjects: 67 subjects experienced a WHO stage 3 or 4 event during follow-up and 483 were censored (did not experience an event during

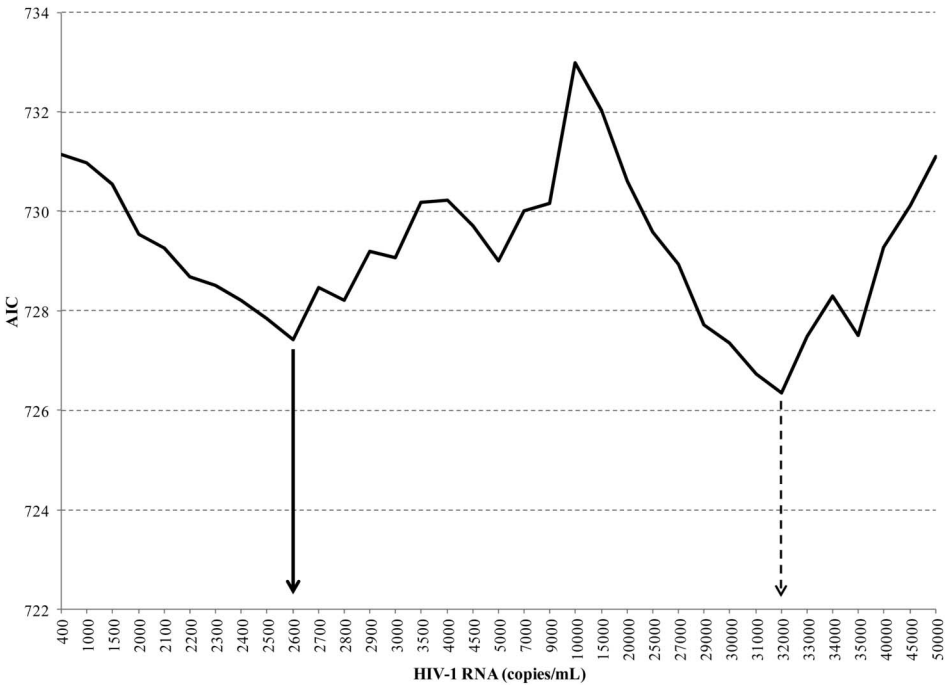

FIGURE 1. Akaike information criterion goodness-of-fit measure plotted as a function of HIV viral load cut-points.

follow-up); there were an additional 22 subjects with WHO events who were excluded from the modeling due to missing values for 1 or more covariates, with most missing CD4 measures.

For each VL cut-point, Table 1 presents (a) the AIC goodness-of-fit measure; (b) the estimated hazard ratio (HR), confidence interval, and *P* value for assessing the contribution of VL to the model; and (c) the *P* values for assessing the contribution of CD4 immunosuppression and hemoglobin to the fitted model. The AIC started at a high point at the VL cut-point of 400 copies per milliliter, declined to the first low point occurring at a VL cut-point of 2600 copies per milliliter before rising again, and then declined to the second low point at a VL cut-point of 32,000 copies per milliliter (Table 1; Fig. 1).

The HR provides an estimate of the instantaneous risk of experiencing a WHO event associated with having a VL at or above the specified cut-point. For example, the HR of 1.61 for the VL cut-point of 400 copies per milliliter indicates that

TABLE 2. Hazard Ratios for Covariates When Using VL Cutoffs of 2600 or 32,000 Copies Per Milliliter in the Model

	2600 VL Cut-Point		32,000 VL Cut-Point	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Visits with VL < cut-point	2550		3766	
Visits with VL ≥ cut-point	1965		749	
VL	2.02 (1.15 to 3.58)	0.015	2.13 (1.25 to 3.66)	0.006
CD4 immunosuppression				
Mild/advanced	2.35 (1.32 to 4.17)	<0.001	2.37 (1.33 to 4.21)	0.003
Severe	3.40 (1.69 to 6.84)		2.98 (1.44 to 6.14)	
None	1.00		1.00	
Hemoglobin	0.76 (0.64 to 0.91)	0.002	0.75 (0.63 to 0.89)	0.001
WHO BMI		0.001		0.004
>2 SD	0.11 (0.02 to 0.51)		0.15 (0.03 to 0.68)	
Normal	0.13 (0.05 to 0.40)		0.16 (0.05 to 0.47)	
<−2 SD	1.00		1.00	
CDC classification				
B	2.01 (0.95 to 4.22)	0.056	1.98 (0.94 to 4.17)	0.046
C	2.41 (1.17 to 4.96)		2.49 (1.21 to 5.10)	
A	1.00		1.00	
Age	0.99 (0.99 to 1.00)	0.14	0.99 (0.99 to 1.00)	0.19

VL, viral load; HR, hazard ratio; CI, confidence interval; CD4, CD4+ T-lymphocyte; WHO, World Health Organization; WHO BMI, WHO-defined body mass index CDC, Centers for Disease Control and Prevention.

subjects who had a VL measure at or above this cut-point were 61% more likely to experience a WHO event as those whose VL did not reach this level. The risk (HR) of WHO events was higher for the VL cutoffs associated with the lowest AIC scores (ie, best fit to the data): subjects with VL at or above the cut-point of 2600 copies per milliliter had a 2.0-fold higher risk (HR) for WHO events compared with those whose VL was below this level ($P = 0.015$); subjects with VL at or above the cut-point of 32,000 copies per milliliter had a 2.1 higher risk (HR) for WHO events compared with those whose VL was below this level ($P = 0.0058$). The HRs for CD4 level, HIV clinical stage, hemoglobin level, body size, and age were very similar when using either VL cutoff of 2600 or 32,000 copies per milliliter in the model (Table 2).

DISCUSSION

Prior analysis of this large cohort of perinatally HIV-infected Latin American children taking HAART for at least 6 months demonstrated that most recent VL greater than the WHO-defined virological failure threshold of 5000 copies per milliliter —but not above the 6-month virological failure threshold of 400 copies per milliliter used by US pediatric treatment guidelines at the time—was an independent predictor of WHO stage 3 and 4 events, even after adjusting for most recent CD4-defined immunosuppression, hemoglobin level, and other cofactors.[6] By repeatedly using the same proportional hazards regression modeling with different VL cutoffs, we were able to demonstrate that 2 distinct VL cutoffs (2600 and 32,000 copies/mL) were the best-fitting models for predicting WHO events among VL cutoffs between 400 and 50,000 copies per milliliter.

It was somewhat expected that this analysis would yield a VL cutoff close to the 5000 copies per milliliter threshold reported in the original analysis. It was surprising, however, that the search for the VL cutoff that added the most independent value for predicting HIV-related clinical events would also yield a second, much higher VL cutoff that had such similar performance characteristics in the model. Furthermore, there was no evidence that the similar performance of these 2 VL cutoffs in the model was due to compensatory differences between the HR for CD4 level, hemoglobin level, age, and other factors included in the model. The VL cutoff of 32,000 copies per milliliter in the present analysis is similar to the 30,000 copies per milliliter threshold used in the Paediatric European Network for Treatment of AIDS 9/Pediatric AIDS Clinical Trials Group 390 (PENPACT-1) study that compared clinical and other outcomes among children randomized to switch to a new HAART regimen at a confirmed VL threshold of 1000 copies per milliliter versus at a threshold of 30,000 copies per milliliter.[9] In that PENPACT-1 study, the clinical outcomes for children who switched at 30,000 copies per milliliter were similar to those for children who switched at the lower VL threshold. The finding in the current analysis of a maximum predictive value for clinical events when children had VL >32,000 copies per milliliter would be consistent with PENPACT-1 findings showing no clinical detriment in waiting until VL of 30,000 copies per milliliter to switch therapy in children.

Other factors besides HIV-related clinical events must also be considered when choosing a VL cutoff for treatment failure or as a trigger to switch to a new HAART regimen in children. In the PENPACT-1 study, there was a trend in children on nonnucleoside reverse transcriptase inhibitor–-based therapy who switched therapy after a VL of 30,000 copies per milliliter to accumulate more resistance mutations to the nucleoside reverse transcriptase inhibitors in the regimen. In contrast, there may be fewer second-line antiretroviral drugs tested or in formulations appropriate for children, limiting the feasibility of making a switch to a new HAART regimen at low VL cutoffs. In resource-rich settings where virological monitoring is routine, virological failure is suspected when adults and children who have been on HAART for at least 6 months have repeatedly detectable VL measurements (≥50–200 copies/mL).[1,2] Assays that detect such low-level VL are unlikely to become available in low-resource settings. However, quantitative RNA assays from dried blood spots have been developed for use in low-resource settings with detection limits that should allow for detecting children who have VL of 30,000 copies per milliliter or even 2600 copies per milliliter.[10] It will now be important to evaluate the clinical predictive value of a broad range of virological cutoffs using data from other cohorts of children on stable HAART to help confirm the best target for virological monitoring in pediatric HIV programs.

ACKNOWLEDGMENTS

The 2011 NISDI PLACES protocol acknowledgment list: the principal investigators, co-principal investigators, study coordinators, data management center representatives, and NICHD staff are as follows: Brazil—Belo Horizonte: Jorge Pinto (principal investigator) and Flávia Faleiro (Universidade Federal de Minas Gerais); Caxias do Sul: Rosa Dea Sperhacke (principal investigator), Nicole Golin (co-principal investigator), and Sílvia Mariani Costamilan (Universidade de Caxias do Sul/Serviço Municipal de Infectologia); Nova Iguacu: Jose Pilotto (principal investigator), and co-principal investigators Beatriz Grinsztejn, Valdilea Veloso, Luis Felipe Moreira, and Ivete Gomes (Hospital Geral Nova de Iguacu—HIV Family Care Clinic); Porto Alegre: Rosa Dea Sperhacke (principal investigator), Breno Riegel Santos (co-principal investigator), and Rita de Cassia Alves Lira (Universidade de Caxias do Sul/Hospital Conceição); Rosa Dea Sperhacke (principal investigator), Mario Ferreira Peixoto (co-principal investigator), and Elizabete Teles (Universidade de Caxias do Sul/Hospital Fêmina); Rosa Dea Sperhacke (principal investigator), Marcelo Goldani (co-principal investigator), Carmem Lúcia Oliveira da Silva, and Margery Bohrer Zanetello (Universidade de Caxias do Sul/Hospital de Clínicas de Porto Alegre); Regis Kreitchmann (principal investigator), Marcelo Comerlato Scotta (co-principal investigator), and Debora Fernandes Coelho (Irmandade da Santa Casa de Misericordia de Porto Alegre); Ribeirão Preto: Marisa M. Mussi-Pinhata (principal investigator), Maria Célia Cervi (co-principal investigator), Márcia L. Isaac, Fernanda Tomé Sturzbecher, and Bento V. Moura Negrini (Hospital das Clínicas da Faculdade

de Medicina de Ribeirão Preto da Universidade de São Paulo); Rio de Janeiro: Ricardo Hugo S. Oliveira (principal investigator) and Maria C. Chermont Sapia (Instituto de Puericultura e Pediatria Martagão Gesteira); Esau Custodio Joao (principal investigator), Maria Leticia Cruz (co-principal investigator), Ana Paula Antunes, and Jacqueline Anita de Menezes (Hospital dos Servidores do Estado); São Paulo: Regina Celia de Menezes Succi (principal investigator) and Daisy Maria Machado (Escola Paulista de Medicina—Universidade Federal de São Paulo); Marinella Della Negra (principal investigator), and co-principal investigators Wladimir Queiroz and Yu Ching Lian (Instituto de Infectologia Emilio Ribas). Mexico—Mexico City: Noris Pavía-Ruz (principal investigator), co-principal investigators Dulce Morales-Pérez and Jorge Gamboa-Cardeña (Hospital Infantil de México Federico Gómez). Peru—Lima: Jorge Alarcón Villaverde (principal investigator) (Instituto de Medicina Tropical "Daniel Alcides Carrión"—Sección de Epidemiologia, Universidad Nacional Mayor de San Marcos), María Castillo Díaz (co-principal investigator) (Instituto Nacional de Salud del Niño), and Mary Felissa Reyes Vega (Instituto de Medicina Tropical "Daniel Alcides Carrión"—Sección de Epidemiologia, Universidad Nacional Mayor de San Marcos). Data Management and Statistical Center: Yolanda Bertucci, Laura Freimanis Hance, René Gonin, D. Robert Harris, Roslyn Hennessey, Margot Krauss, James Korelitz, Kathryn Miller, Sharon Sothern de Sanchez, Sonia K. Stoszek (Westat, Rockville, MD); NICHD: Rohan Hazra (principal investigator), Lynne M. Mofenson, George K. Siberry (Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, Maryland).

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