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Frequent Episodes of Detectable Viremia in HIV Treatment-Experienced Children is Associated with a Decline in CD4+ T-cells Over Time

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Abstract

Background: The clinical consequences of the magnitude and the duration of detectable viremia in HIV-infected children have not been well characterized. We examined the predictors and immunologic consequences over time of frequent episodes of detectable viremia in HIV-infected children followed at Yale-New Haven Hospital.

Methods: We analyzed the CD4+ T-cell and HIV viral load over a 19-year period (1996 to 2013) of 104 HIV-infected children enrolled in the Yale Prospective Longitudinal Pediatric HIV Cohort. Both CD4+ T-lymphocytes and HIV viral load were measured at clinic visits every 3 to 4 months. Longitudinal data analyses using polynomial random coefficients models were conducted to examine overtime changes in CD4+ T-cell counts by frequency of episodes of detectable viremia. Moreover, regression analyses using logistic regression models were used to assess the predictors of frequent episodes of detectable viremia.

Results: One hundred and four (104) HIV-infected children with more than one HIV viral load measurement between 1996 and November 2013 were included in the analysis. Over 80% (N=86) of the children had detectable viral load (HIV RNA viral load ≥50 copies/ml) during more than 50% of their clinic visits. Children with infrequent episodes of detectable viremia had significantly higher CD4+ T-cell counts overtime compared to those with frequent episodes of detectable viremia (P<0.0001).

Conclusions: Both frequency and magnitude of episodes of detectable viremia had effect on CD4+ T-cells. Strict adherence to a treatment goal of undetectable HIV viremia in children is likely to be beneficial.

Keywords: HIV-infected children; CD4+ T-Cell; Transient viremia; Virologic failure; Viral blips

Background

The management of the pediatric HIV epidemic is one of several HIV success stories; despite this, progress has not been uniform worldwide. At the end of December 2013, only 23% of the 3.2 million children estimated to be living with HIV were receiving antiretroviral therapy (ART) and in 2013 alone, 240 000 were newly infected and 190 000 (170000-220000) died of HIV-related causes [1]. ART coverage in children varies from country to country, ranging from 20% to >95% [2]. The goal of ART is to suppress HIV viral replication and restore immune function (i.e., CD4+ T-cell recovery). Uncontrolled HIV viremia leads to depletion of CD4+ T-cells with a concomitant increase in risk for opportunistic infections, AIDS, and death [3-5].

HIV RNA levels vary in individual patients; this variation is greater in children than in adults [6-8]. HIV RNA >500 copies/mL after having formerly achieved virologic suppression is generally accepted as virologic failure [9-11]. However, isolated episodes of plasma HIV RNA >500 copies/mL (i.e., transient viremia) followed by return to levels of viral suppression often observed in adults and children on ART are not considered as virologic failure [9,12,13]. Transient viremia is relatively common among children on ART [12,14]. The underlying mechanisms and clinical consequences of transient viremia are debatable. A number of mechanisms have been implicated, including variable sensitivity of viral load assays [15], random statistical and biological variations [16], opportunistic infections [17], activation of latently infected cells [18], non-adherence to ART [19], and evolution of drug resistant viruses [20,21]. The clinical significance of episodes of transient viremia has evoked mixed reports. While some studies have reported that transient viremia are of no clinical significance [16,22,23], others studies have reported an association between transient viremia and virologic failure [9,24,25], depletion of CD4+ T-cells [26], and emergence of drug resistant viruses [20,21].

However, there is a paucity of data on the clinical significance of transient viremia in children. The higher variability and natural age-associated decline of CD4+ T-cells seen in children may limit the extrapolation of findings of clinical significance of transient viremia in adults to children [27]. The main objectives of our study were to examine the predictors of frequent episodes of detectable viremia and the association between frequent episodes of detectable viremia and CD4+ T-cell changes over time in children.

Methods

Study participants

Longitudinal data were extracted from the Yale Prospective

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Longitudinal Pediatric Cohort study at Yale-New Haven Hospital. The rationale, organization, and recruitment of the subjects for the cohort study have been described previously [28]. In brief, the enrollment of children born to HIV-1-infected mothers in the greater New Haven area in Connecticut began in 1985. All the children either had mothers already known to be HIV-1 seropositive during pregnancy or at the time of delivery or were discovered to be infected with HIV-1 after presenting with an AIDS defining illness. In the analyses contained herein, we used data collected from HIV-infected children from January 1996, when plasma HIV-1 RNA quantification became available, to November 2013. The study protocol was reviewed and approved by Institutional Review Board at Yale School of Medicine. No informed consent was obtained since patient information was anonymized and de-identified prior to analysis.

Study variables: The outcome variables of the study were absolute CD4+ T-cell count and HIV-1 viral load. The predictor variables included, gender, race, age at study entry, age at HIV diagnosis, caregiver type, history of AIDS defining illness, time since HIV diagnosis, CDC clinical staging of HIV infection, and baseline CD4 T-cell count.

Definitions: Detectable viremia was defined as any detectable viral load above the limit of detection of the assay [29]; we used either 50 or 500 copies/mL as the cutoff viral load. The study participants were divided into two categories based on the frequency of episodes of detectable viremia during scheduled clinic visits.

***Infrequent episodes of detectable viremia*:** This category comprised individuals who had detectable viremia during less than 50% of their clinic visits.

Frequent episodes of detectable viremia: This category comprised individuals who had detectable viremia during more than 50% of their clinic visits.

Clinic and follow-up visits

The study participants were seen and examined at the pediatric specialty clinic every three to four months. Since 1996, HIV-1 RNA quantification and CD4+ T- cell counts and percentages were done at every clinic to follow HIV disease progression. The Amplicor Monitor™ test (Roche Diagnostic Systems, Inc., Branchburg, New Jersey, USA) was used for the quantification of the HIV-1 RNA, in accordance with the manufacturer's instructions and the AIDS Clinical Trials Group (ACTG) quality assurance recommendations were followed, as described elsewhere [30]. The assay's detection limit was 500 copies/mL and 50 copies/mL, before 2004 and after 2004, respectively. CD4+ T-cells were quantified by standard dual-platform flow cytometry technology by a certified clinical laboratory at Yale-New Haven Hospital.

Statistical analysis

Descriptive measures were used to summarize the data. Continuous variables were summarized using median and inter quartile range (IQR); categorical variables were summarized using frequency and percent (%). Wilcoxon rank sum and Fisher's exact tests were used to compare continuous and categorical variables, respectively, between subjects with infrequent and frequent episodes of detectable viremia. Regression analyses using logistic regression models were used to estimate the odds of having frequent episodes of detectable viremia. Longitudinal data analyses using polynomial random coefficients models were conducted to examine differences in CD4+ T-cell counts by frequency of episodes of detectable viremia. Two-sided p-values are reported for all the statistical tests used in the analysis.

Results

One hundred and four (104) HIV-infected children with more than one HIV viral load measurement between 1996 and November 2013 were included in the analysis. The demographic and HIV disease characteristics of the study participants are described in Table 1. Fifty four percent were males, 59% were African Americans, and 57% had biological parents as caregivers. The majority of the participants had congenital HIV (93%), moderate to severe CDC classification (65%), and other comorbidities (55%).

The study participants were divided into two categories based on the frequency of episodes of detectable viremia over the study period. The 'infrequent' category comprised individuals who had detectable viremia, viral load above the detection limit of the assay, during less than 50% of their clinic visits. The 'frequent' category comprised individuals who had detectable viral load during more than 50% of their clinic visits. When the limit of detection of the assay was <50 copies/ml, more than 80% (N=86) of the children had frequent episodes of detectable viremia. Table 1 shows the characteristics of the study population stratified by the frequency of episodes of detectable viremia. There were no statistically significant differences in patient characteristics between the two categories.

Figure 1A displays the odds of having frequent episodes of detectable viremia with corresponding 95% confidence intervals.

	All (N=104)	Detectable viremia		P-value
		Infrequent (N=18)	Frequent (N=86)	
Study covariates				
	Median (IQR)			
Baseline CD4 /mm^3	436 (125-725)	550 (26-820)	420 (140-663)	0.48
Baseline VL (Log_{10})	4.79 (4.13-5.32)	4.60 (2.60-5.50)	4.79 (4.20-5.18)	0.85
Age at study entry (years)	7.83 (5.02-11.98)	7.74 (3.70-13.07)	7.83 (5.20-11.76)	0.92
Age at diagnosis (years)	1.79 (0.44-4.91)	2.04 (0.21-5.32)	1.79 (0.52-4.91)	0.82
Time since Diagnosis (years)	5.14 (2.32-7.35)	3.03 (0.60-8.88)	5.42 (2.52-7.32)	0.40
	N (%)			
Gender				1.00
Female	48 (46%)	8 (44%)	40 (47%)	
Male	56 (54%)	10 (56%)	46 (53%)	
Race/Ethnicity				0.80
Black	61 (59%)	10 (56%)	51 (59%)	
Other	43 (41%)	8 (44%)	35 (41%)	
Other Illness				1.00
No	43 (45%)	7 (47%)	36 (44%)	
Yes	53 (55%)	8 (53%)	45 (56%)	
Caregiver				0.79
Parent	59 (57%)	11 (61%)	48 (56%)	
Other	44 (43%)	7 (39%)	37 (44%)	
Mode of transmission				
Congenital	97 (93%)	15 (83%)	82 (95%)	0.10
Other	7 (7%)	3 (17%)	4 (5%)	
CDC Classification				0.37
N: None	14 (13%)	4 (22%)	10 (12%)	
A: Mild	23 (22%)	3 (17%)	20 (23%)	
B: Moderate	30 (29%)	3 (17%)	27 (31%)	
C: Severe	37 (36%)	8 (44%)	29 (34%)	

Table 1: Characteristics of study participants stratified by frequency of detectable viremia.

Although race/ethnicity, baseline CD4+ T-cell count, and time since diagnosis did not reach statistical significance, they were associated with the frequency of detectable viremia. The odds of having frequent episodes of detectable viremia was 34% (OR=1.34, 95% CI: 0.44-4.06) higher in black children compared with children from other races. Moreover, the odds of having frequent episodes of detectable viremia diminished by 21%(OR=0.79, 95% CI: 0.48-1.30) in children with higher baseline CD4+ T-cell count (per 1-SD increase in baseline CD4 counts) compared with children with lower baseline CD4+ T-cell count.

Individual growth models were used to explore participant's longitudinal CD4+ T-cell data over time. Figure 1B displays the empirical growth plot of CD4+ T-cell data with average trend lines (smooth) included for the two detectable viremia categories. The trend lines show that the CD4+ T-cell values for both groups slowly increased from baseline values and decreased at later time points over the study

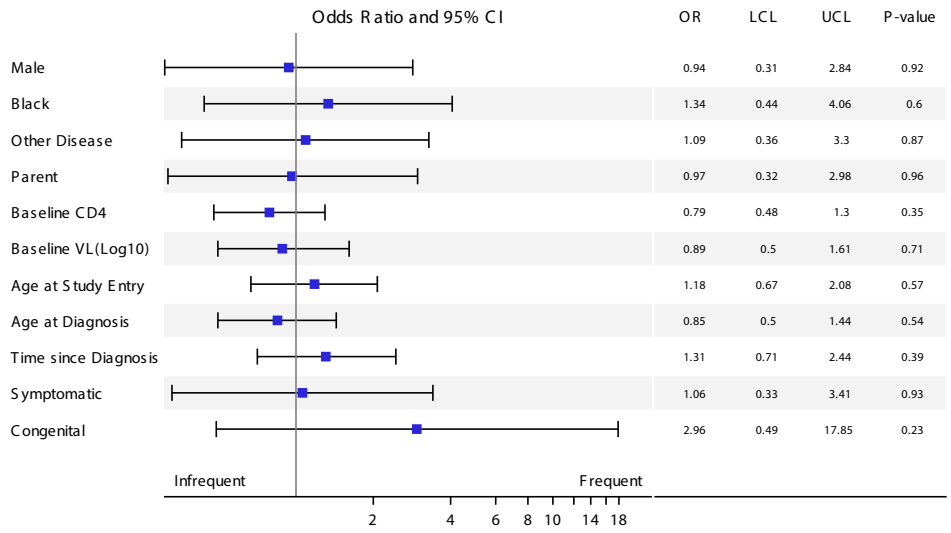

Figure 1A: Risk factors for frequent episodes of detectable viremia among children in Yale-New Haven Hospital.

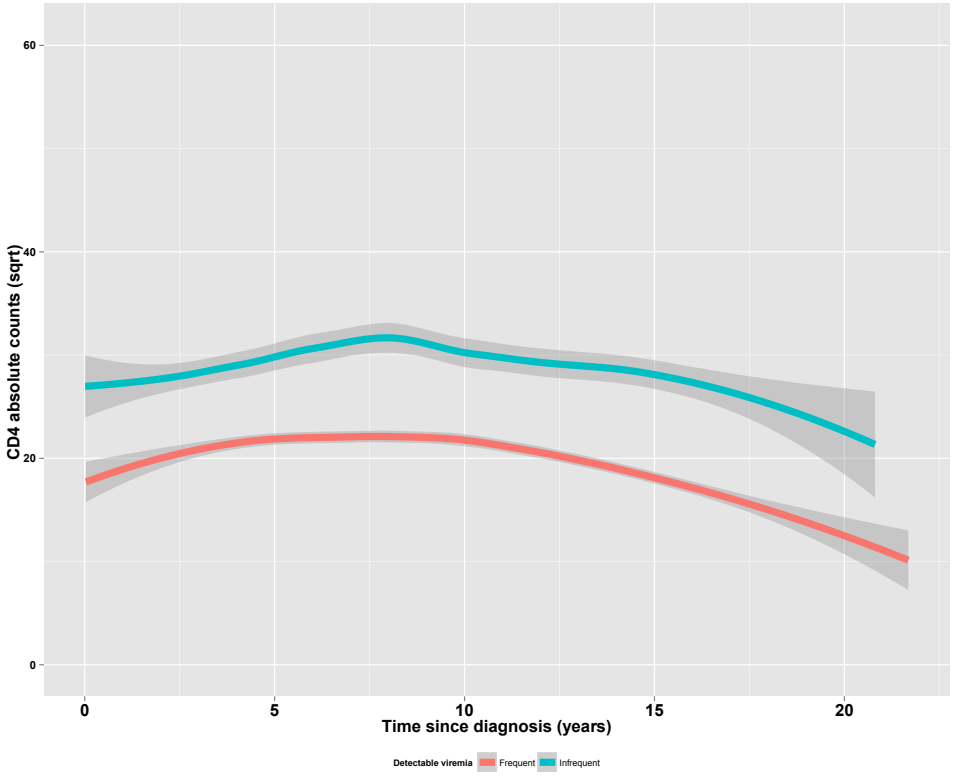

Figure 1B: CD4+ T-cell count trajectories by frequency of detectable viremia status among children in Yale-New Haven Hospital.

period. Since the trend lines were curvilinear, a quadratic random coefficients model was applied. The likelihood ratio test indicated that the quadratic model fits the data better than the linear model (($c^{2=}$461, df=9-6, P<0.0001) and hence results of the quadratic model are reported here. Children with infrequent episodes of detectable viremia had significantly higher CD4+ T-cell counts over time compared with those with frequent episodes of detectable viremia (P<0.0001). The mean square root of the CD4+ T-cell count since HIV diagnosis was 29.13 (95% CI:25.43-32.85) cells/mm^3 in the infrequent group and 20.05 (95% CI: 18.43-21.66) cells/mm^3 in the frequent group. The rate of change in CD4+ T-cell count depended on time in both groups: 2.43-2.45T in the infrequent group and 0.52-0.07T in the frequent group, where, T denotes time from HIV diagnosis. Though the rate of change of the average CD4+ T-cell count over time was not statistically different between the two groups (p=0.10), the rate was higher in the infrequent group. We also conducted a sensitivity analysis using a cutoff of 500 copies/mL. This definition is more reflective of individuals with viremia between 50 to 500 copies/mL. The findings from our sensitivity analyses were identical to that reported above using 50 copies/mL as cutoff (data not shown).

Discussion

We found that frequent episodes of detectable viremia were common in our pediatric cohort. Of the 104 HIV-infected children who were enrolled in the study, more than 80% (N=86) had frequent episodes of detectable viremia (>50 copies/mL) during the course of the study. Moreover, there was an inverse relationship between frequency of detectable viremia and CD4+ T-cell trajectory. The odds of having frequent episodes of detectable viremia were 30% higher in black children compared with children from other races. Higher baseline CD4+ T-cell count was associated with less frequent episodes of detectable viremia. Throughout the study period, children with infrequent episodes of detectable viremia had significantly higher CD4+ T-cell counts.

Our finding of inverse correlation between frequency of episodes of detectable viremia and CD4+ T-cell is consistent with findings from previous studies in adults [26,31-33]. Boufassa et al. reported that viral blips in HIV controllers were associated with a significant decline in CD4+ T-cells [26]. This is consistent with our finding; however, viral blips was defined as any detectable viral load above the limit of detection offered by the assay preceded and followed by an undetectable viral load measurements [29]. Since some of our patients had missing values at random, we did not apply this definition. We rather chose a more liberal and real world definition of any detectable viremia during clinic visits regardless of the interval. Furthermore, Di Mascio et al. reported a negative correlation between frequency of viral blips and baseline CD4+ T-cell counts [31]. Martinez et al. observed that patients with frequent viral blips had lower CD4+ T-cell counts after 12 and 18 months of therapy [32]. They also reported that the frequency rather than the magnitude of the blips was associated with impaired CD4+ T-cell count recovery. We observed that both frequency and magnitude of viral blips had an effect on CD4+ T-cells. Interestingly, other studies have reported that viral blips predate virologic failure [9,24,25]. Virologic failure often results in faster decline of CD4+ T-cells and subsequent AIDS-related clinical events [26]. Our finding, might have resulted from both viral blips and true virologic failures.

Our findings have implications for pediatric ART monitoring in both resource-rich and resource-limited countries. In general, HIV-infected children are less likely than infected adults to achieve full viral suppression on ART [34-36]. Viral load monitoring is not routine in resource-limited countries. The 2013 WHO HIV treatment guidelines define virologic failure as plasma viral load above 1000 copies/mL on two consecutive measurements three months apart in individuals adherent to medication use (www.who.int/hiv). From our finding, this cutoff may be too high, thereby leading to high prevalence of virologic failure and subsequent CD4+ T-cell decline. Moreover, early virologic failure may be missed, resulting in evolution of drug resistant HIV variants in HIV-infected children in sub-Saharan Africa [37,38]. Further studies on the clinical significance of frequency of episodes of detectable viremia in HIV-infected children are needed to inform appropriate cutoffs for virologic failure.

Our study has several strengths compared with previous studies. It is a large population-based study, enhancing its generalizability and it is one of the few studies on frequency of episodes of detectable viremia in children. However, the study has several limitations. First, it spans a period of nineteen years during which time HIV medicine underwent rapid and complex changes in treatment regimens. Since these changes were not envisaged at the inception of the cohort study, critical treatment data are missing, making it difficult to assess the direct impact of the components of various regimens on viremia and CD4+ T-cell counts. Also, viremia during non-adherence may have different consequences from that during complete adherence [9]. Second, our definition of frequency of episodes of viremia is quite liberal; however, in the absence of a consensus on the definition of viral blips, our findings are consistent with other studies using various definitions of viral blips.

Conclusions

In conclusion, strict adherence to ART with the goal of undetectable HIV viremia in children is likely to be beneficial. Frequent viral loads above the detection limit of the assay may lead to unintended persistent viremia with subsequent virologic failure, decline in CD+ T-cell count, and evolution and spread of drug resistant HIV variants.

Authors's contributions

EP, RM, WA, and MG designed the study. RM and MG analyzed the data. EP, RM and MG wrote the article. AG, WA SB revised the article. All authors read and approved the final article.

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