Epstein Barr Virus Infection Is Associated with Compromised Growth in Infants Born to HIV-1 Infected Women

Rebekah Miner

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Jennifer Slyker
Grace John- Stewart

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Abstract

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Rebekah Miner

Chair of the Supervisory Committee:
Assistant Professor Dr. Jennifer Slyker
Department of Global Health

Objective: Infants exposed to and infected with human immunodeficiency virus (HIV) experience high mortality rates, high rates of morbidity, and poor growth. Poor growth is associated with increased mortality rates in infancy as well as impaired neurocognitive development, and poorer educational attainment in the long-term. Other infections acquired in infancy may also be accompanied by acute growth faltering. Epstein Barr (EBV) is a common pathogen that is commonly acquired in infancy and has been causally linked to serious long-term clinical sequelae in children and adults such as mononucleosis and certain malignancies. To date, there have been no studies examining the effect of EBV infection on infant growth. This study examined the association between EBV infection, EBV viral load and infant growth.

Methods: This study utilized data from a prospective observational study conducted in Kenya from 1999-2003. Infants born to HIV-infected mothers (n=125) were followed for 1 year with serial anthropometric measurements. EBV DNA viral loads were measured using quantitative real-time PCR. Infant weight and length were recorded at monthly study visits. Linear mixed effects models were used to determine the effect of EBV infection and EBV viral load on infant's weight-for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ) scores.

Models were adjusted for baseline z-scores for all infants, and additionally for HIV-1 viral load in HIV-infected infants.

Results: The cohort included 75 HIV-infected and 50 HIV exposed uninfected (HIV-EU) infants who were followed for 1 year. EBV infection was associated with altered z-scores in HIV-infected and HIV- EU infants. HIV-infected infants had WAZ on average 0.83 (p=0.01) lower and WHZ scores in average 0.89 (p=0.05) lower than EBV negative infants. HIV- EU infants had HAZ scores on average 1.7 (p=0.04) lower than EBV negative infants. EBV viral load had a statistically significant association with z-scores in HIV-infected infants; each 1-log increase in EBV viral load was associated with a 0.29 lower WHZ score (p<0.001), and 0.28 lower WAZ score (p<0.001). In HIV-EU infants, each 1-log increase in EBV viral load was associated with 0.14 lower WAZ (p=0.05).

Conclusion: EBV may be a contributing factor in impaired growth in HIV-infected and HIV- EU infants. Delaying EBV infection might improve growth during the first year of life for these infants.

Introduction

Numerous studies have established that human immunodeficiency virus (HIV) infection impairs growth in HIV-infected and HIV-exposed infants [1, 2]. It has been estimated that symptomatic HIV infection increases energy needs by 50-100% in children [3]. Adequate nutrition is vital to effective immune function. Common side effects of HIV infection, such as anorexia and lethargy, may lead to decreased intake, resulting in a cycle of malnutrition and worsening HIV infection where growth is compromised [4]. Steady growth through infancy is important because failure to achieve age-standardized growth is associated with increased rates of morbidity and mortality [5].

A recent study by Gompels and her colleagues found that cytomegalovirus (CMV) co-infection was associated with poorer growth in Zambian infants who were born to HIV-infected mothers [6]. CMV is a herpesvirus that is commonly acquired in infancy and is typically asymptomatic in healthy infants; however CMV co-infection is associated with increased neurologic disease and mortality [7] and poor viral containment in HIV-infected infants [8]. The herpesvirus Epstein Barr (EBV) is also commonly acquired in infancy. It is estimated that greater than 90% of the world's population is infected with EBV [9]. Usually EBV infections are asymptomatic and undiagnosed [9]. However, in immunocompromised populations EBV infection can cause malignancy. EBV is a causative factor in many malignancies such as nasopharyngeal carcinoma, gastric cancer, Burkitt lymphoma and in the setting of HIV infection, non-Hodgkin's lymphoma [10].

In east Africa, EBV infection occurs at high rates in infancy. Piriou et al (2012) found an EBV prevalence of about 50% in two rural regions of Kenya in infants followed for their first two years of life [11]. Previous studies have found an EBV prevalence of 98% in Kenyan children aged 1-14 years and 90% by age three in Ugandan children [12, 13]. A recent study conducted in Kenya that examined EBV infection in infants born to HIV-infected mothers found that HIV-infected infants were infected with EBV earlier than uninfected infants. HIV-infected infants often experienced severe systemic and respiratory symptoms during acute EBV infection, including pneumonia [14]. Although EBV incidence in infancy is high in this region, the impact on immediate and long-term infant development is unknown. One study in the United States found that EBV infection was not associated with failure to thrive in HIV-infected infants[15]. However, we are aware of no studies that have evaluated the relationship between EBV infection or EBV viral load and growth.

Understanding the role of viral co-infections in growth and developmental outcomes may yield new strategies to improve the prognosis of children born to HIV-infected women. The aim of this study was to compare age-standardized growth measurements between infants with and without EBV infection and to measure the association between infant EBV viral load and growth.

Methods

Study Design and Follow-Up. The study protocol was approved by the Institutional Review Board of the University of Washington and the Ethics and Research Committee of Kenyatta National Hospital. Women provided written informed consent upon study enrolment. This secondary analysis utilized data from a prospective observational study conducted in Nairobi, Kenya conducted from 1999-2003 that assessed the role of cytotoxic T lymphocytes in the transmission of HIV from mother in infant through breast milk. A detailed description of enrolment, recruitment, clinical assessments and laboratory specimen collection can be found

elsewhere [14]. Women were enrolled before 32 weeks gestation, and were provided with short course zidovudine according to the current standard of care[16]. Women and infants received no other antiretroviral therapy during study follow-up. The infants born to these mothers were classified as either HIV-infected or HIV exposed and uninfected (HIV-EU).

Mother-infants pairs were evaluated at birth, and at monthly clinic visits. HIV-infected infants were followed until they were 24 months old and HIV-EU infants were followed until they were 12 months old. At each visit, infants were examined by a study clinician who recorded anthropometric measurements (length and weight).

Infant HIV and EBV testing. At birth, 1, 3, 6, 9 and 12 months of age a blood sample was taken which was used to determine infant HIV status. HIV was determined by the detection of HIV-1 gag DNA on dried blood spot by PCR [17] or the detection of HIV-1 gag RNA using the GenProbe Assay [18].

EBV was diagnosed using a quantitative real-time PCR assay as previously described [14]. The limit of detection for the assay was 50 EBV DNA copies/ mL of plasma. A subset of infants from the larger cohort were selected for EBV testing; the sample set was powered for the main analysis comparing incidence between HIV-infected and HIV-EU infants. Inclusion criteria for EBV testing were survival to >3 months of age and known HIV status. All HIV-infected infants satisfying theses criteria were selected, along with 50 randomly selected HIV-EU controls.

Statistical Analysis

Stata version 11 (StataCorp, College Station, TX.) was used for all analysis. All analyses presented used alpha=0.05 [19]. Z-scores were calculated from the standardized WHO growth charts using World Health Organization Anthro software [20]. Stunting, wasting and underweight were defined as a z-scores less than -2 SD [21].

To assess the association between EBV infection and infant growth, we used linear mixed effects models (LMM) with random slopes and intercepts. For each growth parameter, we constructed two models. The first used time-updated EBV infection status as a dichotomous predictor variable, which included the interaction term [EBV viral load x time]. The second model used time-updated EBV viral load as a continuous predictor variable and did not include an interaction term. All models were stratified by infant HIV infection and adjusted for baseline z-score. Additionally, models in the HIV-infected stratum were adjusted for the time-updated infant HIV-1 viral load. Infants with late acquisition of HIV (after 1 month of age) were excluded from the LMMs.

To determine the relationship between infant z-scores and time to infant EBV acquisition, we used Cox proportional hazards regression, stratified by HIV status. Infants were censored at study exit or death, whichever came first. We ran the model in two different ways. First we examined the relationship between baseline z-scores and time to EBV infection. Second, we examined the association between time-lagged z-score (using the z-score at the previous visit) and time to EBV acquisition.

Results

Study Participants. The original cohort included 456 singleton or firstborn twins born to HIV-infected mothers. A total of 5 infants were excluded because they were second-born twins and 39 infants were excluded who did not have blood samples taken at birth. A total of 87 acquired HIV

infection during the first year of life. The cohort selected for EBV testing included 125 infants (75 HIV-infected and 50 HIV-EU infants) based on eligibility criteria. The cohort characteristics can be seen in Table 1. By 12 months of age there were 39 EBV infections in the HIV-infected infants and 14 EBV infections in the HIV-EU infants. Although the birth weight and length did not differ between HIV-infected and HIV-EU infants, by 12 months of age, HIV-infected infants had lower HAZ ($p<0.001$), WHZ ($p<0.001$) and WAZ ($p<0.001$) scores compared to their HIV-EU peers. Based on the dramatic difference in growth between HIV-infected and HIV-EU infants, we stratified the remaining analyses by HIV status. As previously described, mortality was very high in this cohort, 50% (n=38) of HIV-infected infants died during follow-up.

Correlates of growth in the cohort. Both maternal and infant factors were associated with infant z scores; we found similar relationships in HIV-infected and HIV-EU infants (data not shown), so data is presented for the entire cohort (Table 2). Maternal weight at 32 weeks was strongly protective for WAZ (η^2=0.033, $p<0.001$), WHZ (η^2 =0.016, p=0.001), and HAZ (η^2=0.033, $p<0.001$). Similarly, BMI was associated with WAZ, WHZ, and HAZ ($p<0.001$ for each). Increasing parity was associated with poorer HAZ, for each additional live birth, HAZ decreased by 0.086 (p=0.03). Married women had infants with higher WAZ (η^2 =0.39, p=0.01) and WHZ (η^2 =0.44, p=0.001) scores. Maternal HIV was inversely correlated with WAZ, WHZ, and HAZ ($P<0.05$ for each), but CD4 percent <20 was only associated with higher WHZ scores (η^2 =0.10, p=0.04).

Infant correlates of growth included prematurity and low birth weight, which was associated with lower WAZ and HAZ scores ($p<0.001$ for each). Infant z scores at birth were also strongly predictive of z scores during the first year of life ($p<0.001$ for baseline and later WAZ, WHZ, and HAZ).

EBV infection and growth. To visualize the growth trajectories of this cohort, we used Lowess and linear fit models stratified by HIV status and time-updated to reflect the infant's current EBV status (Figure 1 and Table 3A). We present final models adjusted for baseline WAZ, HAZ or WHZ, in all infants, and additionally adjusted for infant HIV-1 viral load among the HIV-infected infants. We did not adjust for other co-factors associated with growth because none of these were associated with time to EBV infection [14].

Among HIV-infected infants, WAZ were significantly lower in EBV-infected infants compared to EBV-negative infants (beta=-0.83, 95%CI: -1.44, -0.22). Increasing HIV-1 viral load was also associated with significantly lower WAZ (beta= -0.09, 95%CI -0.16, -0.01). In the HIV-EU infants, WAZ were also lower in EBV-infected infants, (beta=-0.59, 95%CI: -1.32, 0.15), but this did not reach statistical significance.

Among HIV-infected infants, WHZ were significantly lower in EBV-infected infants compared to EBV-negative infants (beta=-0.89, 95%CI: -1.75, -0.02). Among HIV-infected infants every 1-log increase in HIV-1 viral load was associated with a 0.13 lower WHZ score (95%CI 0.02, 0.25). Among the HIV-EU infants, there was a significant difference in the rate of WHZ change over the first year of life, which declined significantly in the EBV-infected infants (beta= -0.14, 95%CI: -0.26, -0.02) but did not change significantly in the EBV-uninfected infants (beta=0.15, 95% CI -0.08, 0.38); The differential between the rates was equivalent to a z-score of 0.14 per month (p=0.03). Among HIV-infected infants every 1-log increase in HIV-1 viral load was associated with a 0.13 lower WHZ score (95%CI 0.02, 0.25).

HAZ were similar between EBV infected and uninfected HIV-infected infants. Among HIV-EU infants, HAZ were significantly lower in EBV-infected infants compared to EBV-negative infants (beta=-1.71, 95%CI: -3.32, -0.09).

Association between infant EBV viral load and growth. For every 1-log increase in EBV viral load WAZ scores decreased by 0.28 (95%CI: -0.42, -0.13) and WHZ scores decreased by 0.29 (95% CI: -0.46, -0.12, Table 3B) in HIV-infected infants. In HIV-EU infants, each 1-log increase in EBV vial load was accompanied by a 0.14 lower WAZ (95% CI: -0.28, 0.00, Table 3B).

Assessing directions of association. To determine whether our findings could be explained by increased susceptibility to EBV among infants with impaired growth, we measured the association between z-scores and time to EBV acquisition. We ran the model two different ways. First we examined the association between baseline z-score and time to EBV infection. Second, we examined the association between z-scores at the time lagged study visit and time to EBV infection. We found no evidence that smaller infants were at greater risk for EBV acquisition; infant z-scores were not associated with time to EBV acquisition in any of the models examined (Table 4).

Discussion

This study provides several new insights into the relationship between EBV infection and infant growth that warrant further investigation. Our study is the first to show that a common infection, EBV is associated with altered z-scores in HIV-infected and HIV-EU infants. We found that among HIV-infected infants, those who were EBV-infected had lower WHZ and WAZ scores. HIV-EU infants who are EBV-infected had lower HAZ scores. EBV viral load was strongly associated with lower WHZ and WAZ scores in HIV-infected infants and WAZ in HIV-EU infants.

To our knowledge, this is the first study that has examined the impact of EBV on growth. A previous study analyzed factors associated with failure to thrive in HIV-infected infants in the United States and found that EBV infection at 18 months of age was not associated with failure to thrive [15]. However, there was a low prevalence of EBV in that cohort with later EBV acquisition compared to the infants in our study. Additionally, the longitudinal assessment of infant z-scores gave us greater power to detect an effect of EBV infection.

There are several reasons why EBV could be associated with impaired growth. As described in HIV infection, there is a metabolic cost of generating an immune response against infections that could be multiplied with concurrent infections (HIV, CMV, EBV) [4]. This may be why we saw z-scores decrease when EBV viral load increased. On top of increased energy requirements, disease symptoms such as nasal congestion, cough or anorexia can lead to decreased nutritional intake. Changes in gut permeability associated with chronic or repeat infections such as HIV, parasitic infections and chronic diarrhea affect nutrient absorption [22, 23]. Over time, malabsorption can lead to impaired growth when essential nutrients are not available.

In this cohort, other co-factors for poor infant growth were similar to those reported in HIV-infected and uninfected infants. Infant growth was associated with indicators of maternal health such as maternal weight, parity and maternal HIV-1 viral load. Prematurity and low birth weight were associated with poor infant growth and are also associated with maternal health [24].

In order to determine whether the association between growth and EBV was explained by increased susceptibility of poorly growing infants to EBV infection, we examined the association between growth and time to EBV infection. Although we cannot completely rule out reverse causality in the associations we observed between EBV infection and growth, we found no evidence to suggest that EBV was acquired earlier in smaller infants. There was also no association between preterm birth or birth weight and time to EBV infection [14].

Our analysis has several strengths and some limitations. Strengths include the longitudinal assessment of growth and EBV infection and the ability to examine potential confounders (maternal and infant viral load). Limitations include the small number of EBV infections in the HIV-EU infants, and the late acquisition of EBV infection in this group (primarily at 9 and 12 months), which reduced power to detect associations between infant EBV and growth. Because our cohort included only HIV-infected women and their infants, we were unable to assess the impact of EBV in growth in HIV-unexposed infants. In this cohort there are also multiple competing risks for bad outcomes (poor growth, EBV infection and mortality) which likely further reduced our effect sizes; suggesting we may have underestimated associations reported in this paper. Finally, because we used EBV DNA to detect infection and not antibodies, it is possible that an EBV infection was missed if the infant successfully suppressed the virus before their next visit when they were tested for EBV. Follow-up analyses will include EBV serology to further refine our EBV exposure variable.

This study suggests EBV infection might be a contributing factor to poor growth in HIV-infected and HIV-EU infants. Vaccination, delaying EBV infection, or reducing EBV viral load might improve growth during the first year of life for these infants. More research is needed to understand the relationship between EBV infection and growth in infants born to HIV-infected women; and it will be important to determine whether EBV is associated with growth changes in HIV-unexposed infants.

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Table 1: Cohort Characteristics

	HIV-infected (n=75)	**HIV-EU (n=50)**
Birth Weight (kg)	3.2 (3, 3.4)	3.1 (2.9, 3)
Birth Length (cm)	49 (46,52)	49 (47, 50)
Time in FU (in months)	12 (6,24)	12 (10,12)
Number of Visits:	5 (3,5)	4 (4,5)
Number of times tested for EBV	6 (4,8)	5 (4,5)
EBV positive by PCR	61% (46)	22% (11)
by 3 months old	8% (6)	4% (2)
by 6 months old	27% (20)	4% (2)
by 12 months	52% (39)	22% (11)
Median weight-for-age z-scores at 12 months	-1.25 (-2.7, -0.86)	-0.19 (-1.11, 0.51)
Median weight-for-height z-scores at 12 months	-0.74 (-1.9, 0.35)	0.35 (-0.51, 1.1)
Median height-for-age z-scores at 12 months	-2 (-2.83, -1.6)	-0.9 (-1.9, -0.02)
Mortality	50% (38)	2% (1)

Notes: Values are median,(IQR) or percentage,(n)

Table 2: Correlate of infant growth

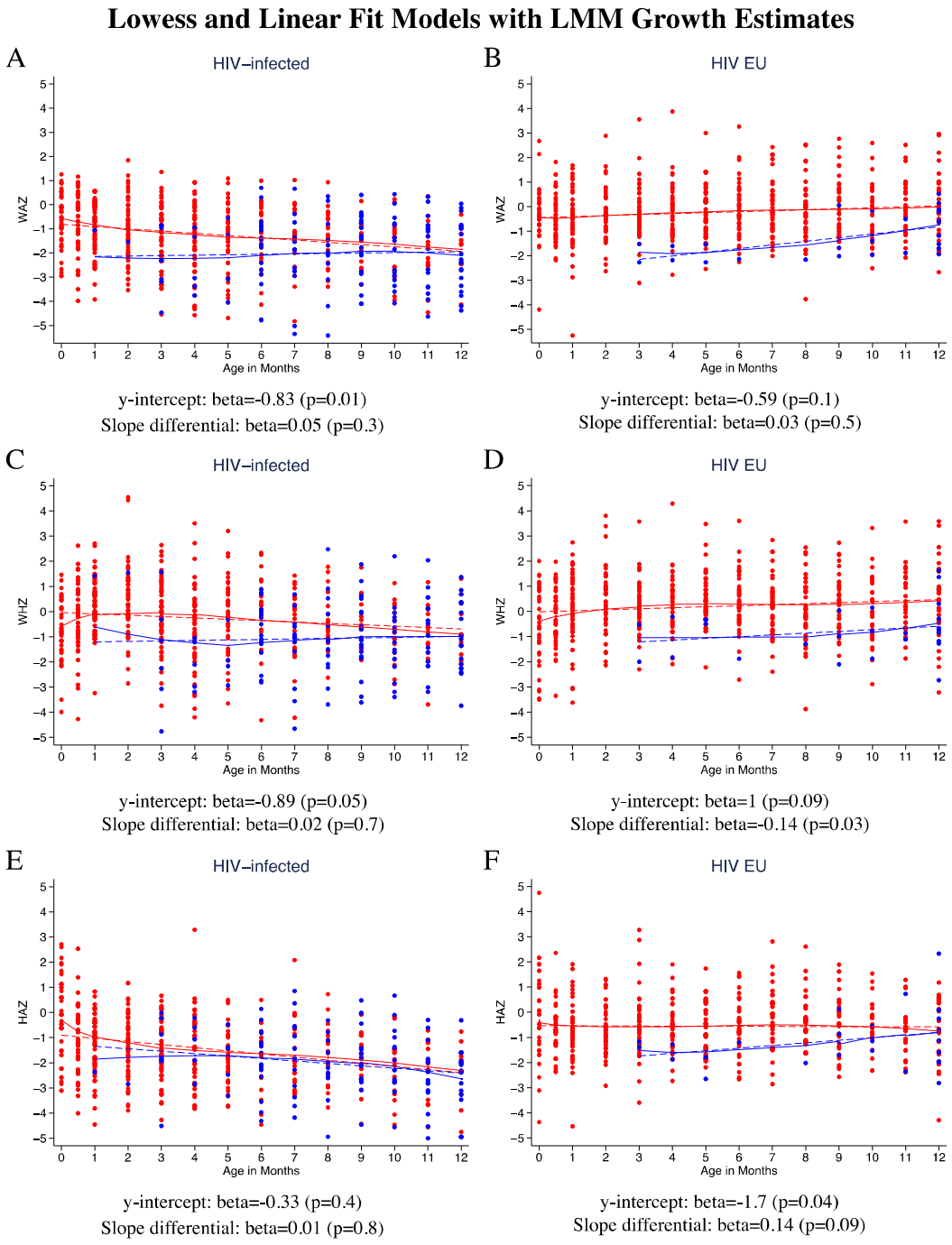
Covariate	N	WAZ Beta, p	WHZ Beta, p	HAZ Beta, p
Maternal characteristics				
Age	462	-0.0098 [-0.032, 0.012], p=0.4	-0.0040 [-0.023, 0.015], p=0.7	-0.0071 [-0.029, 0.015], p=0.5
Weight at 32 weeks gestation	448	0.033 [0.023, 0.042], p<0.001	0.016 [0.0066, 0.025], p=0.001	0.033 [0.023, 0.043], p<0.001
BMI at 32 weeks gestation	443	0.082 [0.051, 0.11], p<0.001	0.058 [0.030, 0.087], p<0.001	0.061 [0.029, 0.093], p<0.001
Parity	458	-0.073 [-0.15, 0.0034], p=0.06	-0.037 [-0.10, 0.029], p=0.3	-0.086 [-0.16, -0.010], p=0.03
Married	456	0.39 [0.091, 0.68], p=0.01	0.44 [0.18, 0.70], p=0.001	0.070 [-0.23, 0.37], p=0.6
HIV viral load	456	-0.12 [-0.18, -0.058], p<0.001	-0.082 [0.0056, 0.16], p=0.04	-0.15 [-0.23, -0.063], p=0.001
CD4 percent <20	462	-0.032 [-0.087, 0.024], p=0.3	0.10 [0.0072, 0.19], p=0.04	-0.071 [-0.18, 0.035], p=0.2
Infant characteristics				
Preterm*	377	-1.2 [-1.5, -0.90], p<0.001	-0.15 [-0.45, 0.15], p=0.3	-0.98 [-1.3, -0.65], p<0.001
Low birth weight**	367	-2.2 [-2.6, -1.8], p<0.001	-0.55 [-0.95, -0.15], p=0.007	-1.8 [-2.2, -1.4], p<0.001
Small for gestational age*	348	-1.3, [-1.6, -0.98], p<0.001	-0.55 [-0.87, -0.23], p<0.001	-1.1 [-0.83, -0.60], p<0.001
WAZ birth	455	0.86 [0.81, 0.91], p<0.001	0.28 [0.20, 0.36], p<0.001	0.74 [0.67, 0.81], p<0.001
WHZ birth	454	0.26 [0.21, 0.32], p<0.001	0.38 [0.33, 0.42], p<0.001	0.026 [-0.036, 0.088], p=0.4
HAZ birth	455	0.20 [0.15, 0.24], p<0.001	-0.032 [-0.073, 0.0077], p=0.1	0.39 [0.35, 0.42], p<0.001

Notes. Includes all infants, estimates were similar in HIV-infected and HIV-EU infants.

*Among infants with Dubowitz assessed <3 days of birth.

**Among infants with weight assessed <24 hours of birth.

Figure 1: Longitudinal changes in infant growth in EBV-infected and EBV-uninfected infants



Y- intercept and difference in slope estimates from linear mixed effects models adjusted for baseline z-scores and infant HIV-1 viral load in HIV-infected infants.
All lowess and linear fit models adjusted for time-updated HIV-1 viral load in HIV-infected infants.

Table 3: Associations between EBV infection, EBV viral load, and infant growth.

	HIV-infected*		HIV-EU	
	Beta(95%CI)	P value	Beta(95%CI)	P value
Model A: Time-updated EBV infection status				
Weight for Age				
Mean difference for EBV+	-0.83 (-1.4, -0.22)	0.01	-0.59 (-1.3, 0.15)	0.1
Slope differential	0.05 (0.03, 0.13)	0.3	0.03 (-0.05, 0.1)	0.5
HIV-1 viral load	-0.09 (-0.16, -0.01)	0.02	NA	NA
Weight for Height				
Mean difference for EBV+	-0.89 (-1.8, -0.02)	0.05	1.04 (-0.18, 2.2)	0.09
Slope differential	0.02 (-0.09, 0.13)	0.7	-0.14 (-0.26, -0.02)	0.03
HIV-1 viral load	0.13 (0.02, 0.25)	0.02	NA	NA
Height for Age				
Mean difference for EBV+	-0.33 (-1.1, 0.45)	0.4	-1.7 (-3.3, -0.09)	0.04
Slope differential	0.01 (-0.09, 0.1)	0.8	0.14 (-0.02, 0.3)	0.09
HIV-1 viral load	-0.07 (-0.16, 0.02)	0.2	NA	NA
Model B: Time-updated EBV viral load				
Weight-for-age	-0.28 (-0.42, -0.13)	<0.001	-0.14 (-0.28, 0.00)	0.05
Weight-for-height	-0.29 (-0.46, -0.12)	<0.001	-0.01 (-0.34, 0.32)	0.9
Height-for-age	-0.12 (-0.24, 0.01)	0.06	-0.21 (-0.49, 0.07)	0.1

*Adjusted for baseline z-scores and time-updated infant HIV-1 viral load

Table 4: Associations between infant z-scores and time to EBV acquisition.

	Baseline Visit HR(CI), p value	**Time-lagged Prior Visit** HR(CI), p value
HIV-infected		
Weight-for-age	1.1 (0.8-1.7), 0.7	1(0.7-1.3), 0.9
HIV viral load	1.2(0.7-1.9), 0.6	1.2(0.7-2.1), 0.5
Weight-for-height	1(0.8-1.3), 0.9	1(0.8-1.4), 0.8
HIV viral load	1.18(0.7-2), 0.5	1.2(0.7-2.2), 0.5
Height-for-age	1.2(1-1.5), 0.1	1(0.7-1.3), 0.9
HIV viral load	1.1(0.7-1.8), 0.3	1.2(0.7-2.1), 0.5
HIV-EU		
Weight-for-age	1.1(0.7-1.8), 0.7	0.7(0.4-1.2), 0.3
Weight-for-height	0.9(0.6-1.3), 0.4	0.8(0.5-1.3), 0.4
Height-for-age	1.1(0.8-1.3), 0.6	0.8(0.5-1.4), 0.4