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Young age at start of antiretroviral therapy and negative HIV antibody results in HIV-infected children when suppressed

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Abstract

Background—Negative results on standard HIV antibody tests have been described among HIV-infected children suppressed on antiretroviral therapy (ART) started early in life. Here we describe the frequency and predictors of this phenomenon in a well-characterized cohort of treated children.

Methods—We selected samples from 103 HIV-infected children who started ART 14 months of age and from 122 children who started 6 months of age followed as part of two sequential clinical trials in Johannesburg, South Africa. Children had attained viral suppression on ART and had received ART for between 3 and 6.4 years (mean 4.3 years) when tested for HIV antibody using a standard ELISA (Genescreen™ HIV1/2 version 2; Bio-rad).

Results—Only children 6 months of age when ART was started had negative antibody results when tested after suppression on ART several years later. Negative or low-positive antibody results were observed in 40.0%, 37.0% and 27.8% of children starting ART <2 months of age, or starting during month 2 or 3, respectively. This dropped to 5.9%, 3.5%, and 5.3% if ART was started during month 4, 5, and 6, respectively. Higher CD4 percentage prior to ART initiation and no recorded intermittent viremia also predicted negative antibody results.

Conclusion—Testing negative on standard HIV antibody tests occurs fairly commonly among HIV-infected children who started ART 3 months of age and are virally-suppressed. It would be

prudent in clinical practice to avoid HIV antibody tests among virally-suppressed, early-treated children to prevent unnecessary confusion.

Introduction

HIV antibody tests are considered to be diagnostic in adults and older children but cannot be used in infancy for diagnosis. This is because of transplacental passage of maternal HIV antibodies which may persist in the young child at detectable levels for up to 18 months or longer.¹ Before this age, these tests cannot distinguish the child's from the mother's HIV infection. After this age, HIV antibody tests are used routinely for diagnosis in children, in the same way that they are used in adults, with the typical expectation that antibody status does not revert to negative after a positive result.²

Thus the reports of virologically-confirmed, HIV-infected children suppressed on antiretroviral therapy (ART) who have negative HIV antibody tests are intriguing.³ An early U.S. report described 16/17 infected infants initiating ART at 15 days to 3 months of age becoming antibody negative by 16 months.⁴ Five of 12 early-treated children in Belgium and 4 of 6 in Italy have also been reported to be persistently antibody negative once suppressed.⁵⁻⁷ The so-called Mississippi baby who started ART within 30 hours of birth and who maintained viral control for more than two years after ART was stopped also had negative HIV antibody results thus reviving interest in this issue.⁸ The recent case reports of early-treated children have also reported negative HIV antibody results during suppressive ART.⁹⁻¹¹

In the clinical setting, a negative HIV antibody test in an ART-treated child raises a variety of concerns for clinicians and parents. Virologic and diagnostic testing history would need to be reviewed to determine whether the child was initially misdiagnosed. If indeed the child is confirmed to be HIV-infected, then clinicians would need to explain to parents the significance of the negative antibody result. This would most likely include clarification that the antibody test result does not mean that the child is no longer HIV-infected and emphasis on the continuation of the child's ART. There are concerning anecdotal reports of health care workers stopping ART in children testing antibody negative based on mistaken assumptions.¹² Thus, better understanding of the frequency of this phenomenon in clinical populations, particularly in sub-Saharan Africa, is important to ensure appropriate clinical management for HIV-infected children. Existing published reports provide limited information about the frequency of HIV antibody negativity in ART-treated children and are largely based on small numbers of highly-select children from academic centers in North America and Europe.

Here we describe the frequency and predictors of testing HIV antibody negative in a well-characterized cohort of HIV-infected, ART-treated children in Johannesburg, South Africa.

Methods

We selected samples in two rounds from HIV-infected children who had started therapy before two years of age followed as part of two sequential clinical trials at Rahima Moosa Mother and Child Hospital in Johannesburg, South Africa.^{13,14} These trials were approved

by the Institutional Review Boards of Columbia University and the University of the Witwatersrand. The child's guardian provided signed informed consent. All children had been diagnosed as HIV-infected on at least one standard qualitative HIV-1 PCR confirmed on at least one quantitative viral load test. All children had been exposed to nevirapine used for prevention of mother-to-child transmission (PMTCT), given to the mother, to the child, or both. All children met clinical or CD4 criteria to initiate ART which included World Health Organization stage III or IV disease, CD4 percentage <25 if younger than 12 months or <20 if older than 12 months, or recurrent (> 2/year) or prolonged (> 4 weeks) hospitalization for HIV-related complications. Guidelines that advised initiating ART regardless of CD4 and clinical status were not yet in place when these children initiated therapy. All children were initiated on a protease inhibitor-based regimen, mostly ritonavir-boosted lopinavir (LPV/r). Stavudine and lamivudine were the most widely used backbone regimens.

For round one, we selected samples from children enrolled in a clinical trial of switching from LPV/r-based to nevirapine-based regimens after suppression on LPV/r within the first year of treatment.¹³ We selected children from the LPV/r control arm of this trial who were

14 months of age when ART was originally initiated and who remained in long-term follow-up and were enrolled into a second trial.¹⁴ The second trial involved randomization to remain on LPV/r or to switch to efavirenz, and eligibility criteria included being virally suppressed to <50 copies/ml at enrollment. We selected one sample per child from the 24 weeks post-randomization visit in the second trial. This sample could be located for 49/53 children. In round one, we also selected children from the nevirapine switch arm of the first trial who were 14 months of age when ART was originally initiated and who remained in long-term follow-up either without viral failure and still on nevirapine or if having failed nevirapine were now on LPV/r at the time the second trial began. These children were not eligible for the second trial but followed as part of an observational protocol for 24 weeks with those still on nevirapine switched to efavirenz. Fifty four of 57 of the 24 week samples collected for these children could be located. In sum, round one consisted of 103 HIV-infected children who had started ART 14 months of age and who had been well-maintained on ART for several years before testing.

These samples were tested for HIV antibody at the National Institutes of Communicable Diseases in Johannesburg using a standard enzyme immunoassay (EIA) (Genescreen™ HIV1/2 version 2; Bio-rad). Consistent with manufacturer's instructions, all those with optical density (OD) readings below the standard were defined as “negative” and those with OD readings above the test standard and above one OD unit were defined as “positive.” Those with OD readings below one but above the test standard were classified as “low-positive”.

Since we observed no negative or low-positive results in any child who started ART after 6 months of age in round one, for round two we selected only children who were up to 6 completed months of age or younger (i.e. 6 months) at the time of starting therapy. We selected all children enrolled in the second trial and not already tested in round one, who met this age criterion.¹⁴ A sample from the 24 week post-randomization visit could be located for 122/126 children who met these criteria.

Clinical and laboratory data, collected as part of each trial, were used to describe the populations in terms of their pre-treatment characteristics, e.g. age at starting ART, pre-treatment viral load, CD4 count/percentage, weight-for-age etc., as well as characteristics at the time of antibody testing. We also reviewed the study databases to summarize the viral control history based on all available viral load measurements.

Proportions were compared between groups with chi-square tests, and continuous measurements were compared between groups using t-tests. Multivariable logistic regression was used to investigate independent effects of the predictors. Analysis was conducted using SAS software (Cary, NC).

Results

The 103 children tested in round one had started ART at a mean of 7.8 months of age (minimum 2.2, maximum 14.9 months) and were a mean of 5.4 years of age when tested for HIV antibodies, having been on ART for the past 4.8 years. The 122 children tested in round two had started ART at a mean of 3.9 months of age (minimum 0.8, maximum 6.9 months) and were a mean of 4.3 years of age when tested for HIV antibodies, having been on ART for the past 4 years (Table 1). Although eligibility for the trial included having a viral load <50 copies/ml, at the time of antibody testing 2.4% had a viral load >50 copies/ml and 43.4% had one or more measurement >50 copies/ml recorded after initial suppression. Other characteristics of these children at the time of antibody testing and at the time of starting ART are shown in Table 1.

Of 103 children tested in round one, 5 (4.9%) had undetectable and 2 (1.9%) had low-positive antibody reactivity. All of the children with negative/low results in round one were under 5 months of age at ART start (mean 3.6 months, range 2.2 to 4.9 months). Among the 48 children 6 months at ART start in round one, 5 (10.4%) and 2 (4.2%) had negative and low-positive results respectively. Among 55 children who started ART 7 to 14 months of age, all antibody tests were positive. There was no difference in antibody reactivity between those who had and had not ever experienced nevirapine-based therapy.

In round two, among 122 children who were 6 months of age at ART start, 23 (18.9%) had undetectable and 3 (2.5%) had low-positive antibody results when tested on treatment about 4 years later. The mean age at ART start in those with negative/low-positive results in round two was 2.8 months (range 1.4 to 6.4 months).

Four children had viral loads >1000 copies/ml at the time of antibody testing. All four had positive antibody results. Two of three children with viral load 50–1000 copies/ml at the time of antibody testing had positive results. We elected to exclude these 7 children from subsequent analyses.

OD measurements on the HIV antibody test were strongly related to age at starting ART (Figure 1). The mean OD measurement (SD) for children who started ART under 6 months of age was 3.5 (1.8) compared to 4.7 (0.9) if ART started at 7–14 months of age ($p<0.001$).

Negative or low-positive results on the antibody test were observed in 40.0%, 37.0% and 27.8% of the children who started ART under 2 months of age, or during their second or third month of life, respectively. This dropped to 5.9%, 3.5%, 5.3% if ART was started during month 4, 5, or 6, respectively. None of the children who started ART 7–14 months of age had negative or low-positive antibody tests (Figure 2).

To examine potential predictors of testing antibody negative or low several years after starting ART, we combined data on 165 children from rounds one and two who were 6 months of age when ART started and who were currently suppressed to < 50 copies/ml on ART (Table 2). Age at the time of antibody testing and duration of ART were not related to antibody status, nor was current ART regimen or current CD4 status. A history of viremia >1000 copies/ml was significantly associated with being antibody positive (Table 2). If a cut-off of >50 copies/ml was used, the association was still in the same direction but was attenuated and was no longer significant. It should be noted that a history of intermittent viremia in the 50–1000 range did not preclude testing negative/low-positive and a third (11/32) of those with negative/low-positive antibody status had a history of viremia >50 copies/ml while on ART.

Pre-treatment viral load and weight-for-age were not associated with antibody outcome but a higher CD4% pre-treatment was associated with greater likelihood of testing antibody negative/low-positive at a later stage (Table 2). After adjustment for age at ART start as a continuous variable (odds ratio [OR]=0.51 95% CI: 0.36, 0.72), each percentage point of CD4% pre-treatment was associated with an increase in the likelihood of testing antibody negative/low-positive years later when suppressed on ART (OR=1.06; 95% CI: 1.01, 1.11).

Discussion

The absence of a positive HIV antibody result on a standard EIA assay was a fairly common finding occurring in 34% of HIV-infected children who had started ART 3 months of age and who were virally-suppressed. All of these children had started ART for clinical or immunologic criteria and had received standard protease-inhibitor-based regimens as their first-line treatment. Although these children were part of clinical trials, they are likely to be reasonably representative of the patient population of well-functioning HIV treatment programs in South Africa.

We recommend that HIV antibody tests for diagnosis in treated children be avoided. The potential for confusion among health care workers and parents is great. “False negative” results are not simply a result of limitations of the rapid HIV antibody tests and occur with standard EIA assays as well.^{12,15,16} Greater attention should be paid to ensuring that the initial diagnosis prior to initiation of ART is based on adequate virological tests. If circumstances require confirmation at a later stage, qualitative HIV DNA PCR tests are to be preferred. Similar recommendations have been made previously based on similar results.¹⁷

We found a significant association between age at starting treatment and failure to detect HIV antibody several years later when suppressed on ART. Although a third of children who started ART 3 months of age had negative or low-positive HIV antibody results, <5%

who started ART 4–6 months of age had these results. All children who started ART at 7 months of age or older tested HIV antibody positive. Younger age at starting ART in children has been found to be strongly related to the size of the viral reservoir.^{18–21} One study reported that reduced reservoir size was observed in children who suppressed by 12 months of age.¹⁸ Age at viral control is a more complex characteristic to compare between groups as it depends on the frequency of viral load testing once ART is started as well as the age when ART is started. To the extent that an absence of an HIV antibody response indicates a smaller viral reservoir, our data suggest that three months of age for ART initiation may be an upper limit after which benefits of early ART may be attenuated. We saw a slight trend towards increasing proportions with negative/low-positive antibody results among the group starting ART below three months of age. However, the youngest age at starting ART in our cohort was 3 weeks of age and a sizable proportion of the cohort had recorded intermittent viremia after suppression, perhaps explaining why the proportion testing negative was not higher than has been reported in infants starting ART within days of birth.^{5–11}

No child with a history of viremia >1000 copies/ml after initial suppression had a negative/low-positive HIV antibody test. This is consistent with the premise that early ART rapidly removes the antigenic stimulation needed to sustain an HIV-specific antibody response.^{18–22} However, almost a third of those children with negative antibody responses had a history of viremia in the 50–1000 copies/ml range. It will be important to study the initial development of HIV-specific antibodies of different types in early treated infants to determine whether it is primary ontogeny of these responses that is affected or whether the influence occurs later leading to decline of responses over time. In infants this is challenging to study as transplacental passage of maternal antibody complicates distinguishing infant-specific responses. In adults with incident HIV infection treated close to the time of presumed infection, both the absence of the development of an HIV antibody response and the reversion from antibody positivity to negativity has been described.^{23,24} However, the adult studies are difficult to generalize to the infant situation given, amongst other reasons, the variability of definitions of acute and recent infections.

A higher CD4 percentage prior to treatment start also correlated with a higher likelihood of negative HIV antibody responses in our cohort. This association was independent of age at starting ART. Our cohort started ART in an era before universal treatment for all infants was recommended, however, criteria for starting ART were broad allowing wide latitude for starting infected infants on treatment. Our findings suggest that those children with less advanced immune damage prior to treatment start were more likely to have negative HIV antibody responses later. More recent cohorts when treatment is started regardless of clinical or immunologic staging may thus observe higher rates of HIV antibody negativity.

A limitation of our study is that it included few children who started ART within the first month of life and none who started within days of birth. However, it includes a large number who started within one to three months of age, providing a reasonably precise estimate of the likelihood of testing antibody negative years later when suppressed on ART. Although samples were selected from children participating in a clinical trial, data on key parameters particularly prior to starting treatment were missing for some, limiting our capacity to

examine predictors. We used only one standard method for measuring HIV antibody with the goal of describing the frequency of negative and low-positive results. Thus we cannot comment on the performance of other assays, including rapid antibody tests.

By describing the frequency of testing HIV antibody negative in known HIV-infected infants suppressed on ART, we provide useful data to inform clinical practice. It would be prudent to avoid use of HIV antibody tests in children who initiated ART under 6 months of age as the potential for misinterpretation of the significance of the result is great. Much remains to be understood about how early treatment influences HIV latency in infancy.²⁵ Our results help provide a clinical context in which to further investigate these newly-emerging issues in HIV pathogenesis thereby to strengthening HIV prevention and treatment for children.

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Figure 1. Optical density on HIV antibody test by age at antiretroviral therapy start (months) among 218 HIV-infected children suppressed on treatment for 4–5 years

Note: Excluded those not suppressed (N = 7)

Figure 2.

Prevalence of negative and low-positive HIV antibody results by age at antiretroviral therapy (ART) start (months) among 218 HIV-infected children suppressed on ART for ~4 years.

Table 1

Characteristics pre-treatment and at the time of antibody testing of 225 children selected in two rounds from antiretroviral-treated HIV-infected children in Johannesburg, South Africa

Characteristic	Round 1 14 months at ART start (n = 103)	6 months at ART start (n=48)	7–14 months at ART start (n=55)	Round 2 6 months at ART start (n=122)	P-value round 1 vs. 2	P-R 1 6 months vs. R 2
Sex, N (%)						
Male	54 (52.4)	20 (41.7)	34 (61.8)	57 (46.7)	0.394	0.551
Female	49 (47.6)	28 (58.3)	21 (38.2)	65 (53.3)		
Age at ART initiation (months)						
Mean $\pm$ SD	7.8 $\pm$ 3.7	4.3 $\pm$ 1.2	10.8 $\pm$ 2.1	3.9 $\pm$ 1.6	<0.001	0.068
Min, Max	2.2, 14.9	2.2, 6.9	7.1, 14.9	0.8, 6.9		
Age when tested for AB (years)						
Mean $\pm$ SD	5.4 $\pm$ 0.8	5.0 $\pm$ 0.6	5.8 $\pm$ 0.8	4.3 $\pm$ 0.5	<0.001	<0.001
Min, Max	3.7, 7.5	3.7, 6.0	4.4, 7.5	3.5, 5.6		
Time on ART until AB testing (years)						
Mean $\pm$ SD	4.8 $\pm$ 0.7	4.7 $\pm$ 0.5	4.9 $\pm$ 0.7	4.0 $\pm$ 0.5	<0.001	<0.001
Min, Max	3.4, 6.4	3.4, 5.7	3.7, 6.4	3.0, 5.4		
HIV RNA at time of AB testing, N (%)						
50	99 (96.1)	46 (95.8)	53 (96.4)	119 (97.5)	0.502	0.257
51–1000	1 (0.97)	0 (0.0)	1 (1.8)	2 (1.6)		
>1000	3 (2.91)	2 (4.2)	1 (1.8)	1 (0.8)		
CD4 percentage at time of AB testing						
Mean $\pm$ SD	36.2 $\pm$ 7.4	37.2 $\pm$ 7.5	35.4 $\pm$ 7.3	34.9 $\pm$ 7.7	0.200	0.089
Min, Max	18.7, 52.9	22.3, 52.9	18.7, 49.8	17.5, 56.9		
WAZ at time of AB testing						
Mean $\pm$ SD	−0.56 $\pm$ 1.0	−0.67 $\pm$ 0.9	−0.46 $\pm$ 1.0	−0.89 $\pm$ 1.0	0.009	0.164
Min, Max	−4.4, 2.2	−2.29, 1.2	−4.4, 2.2	−3.0, 1.4		
Regimen at time of AB test, N (%)						
LPV/r-based	34 (33.0)	12 (25.0)	22 (40.0)	60 (49.2)	0.021	0.009
EFV-based	68 (66.0)	35 (72.9)	33 (60.0)	60 (49.2)		
Other	1 (1.0)	1 (2.1)	0 (0.0)	2 (1.6)		
Highest HIV RNA (copies/mL) after suppression before AB testing, N (%)						
Always 50	36 (35.0)	17 (35.4)	19 (34.6)	69 (56.6)	<0.001	0.042
51–1000	36 (35.0)	21 (43.8)	15 (27.3)	38 (31.2)		
>1000 copies/mL	31 (30.0)	10 (20.8)	21 (38.2)	15 (12.3)		

Characteristic	Round 1 14 months at ART start (n = 103)	6 months at ART start (n=48)	7–14 months at ART start (n=55)	Round 2 6 months at ART start (n=122)	P-value round 1 vs. 2	P-R 1 6 months vs. R 2
Birthweight (grams), Mean $\pm$ SD	3043 $\pm$ 607	2964 $\pm$ 520	3115 $\pm$ 672	2738 $\pm$ 602	<0.001	0.025
Pre-treatment WAZ, Mean $\pm$ SD	-1.81 $\pm$ 1.7	-1.98 $\pm$ 1.9	-1.66 $\pm$ 1.6	-2.36 $\pm$ 1.5	0.048	0.263
Pre-treatment HIV RNA (copies/mL), N (%)						
<100,000	12 (13.6)	5 (12.2)	7 (14.9)	13 (12.3)	0.450	0.173
100,000–750,000	21 (23.9)	7 (17.1)	14 (29.8)	34 (32.1)		
750,000	55 (62.5)	29 (70.7)	26 (55.3)	59 (55.7)		
Pre-treatment CD4 percentage, Mean $\pm$ SD	20.8 $\pm$ 9.6	24.7 $\pm$ 8.8	17.3 $\pm$ 8.9	24.6 $\pm$ 11.2	0.010	0.952
Pre-treatment CD4 percentage, N (%)						
<10	7 (7.5)	0 (0.0)	7 (14.3)	9 (7.7)	0.010	0.172
10–14.9	22 (23.4)	7 (15.6)	15 (30.6)	14 (12.0)		
15–19.9	27 (28.7)	12 (26.7)	15 (30.6)	19 (16.2)		
20–24.9	9 (9.6)	5 (11.1)	4 (8.2)	20 (17.1)		
25	29 (30.9)	21 (46.7)	8 (16.3)	55 (47.0)		
Pre-treatment CD4 count, Mean $\pm$ SD	1119 $\pm$ 665	1217 $\pm$ 678	1029 $\pm$ 647	1486 $\pm$ 850	<0.001	0.060

Note: ART – Antiretroviral therapy (ART); AB = HIV antibody; IQR = Interquartile range (25th percentile, 75th percentile); SD = standard deviation; WAZ = weight-for-age Z-score. Numbers do not always add up to totals due to missing data.

Table 2

Risk factors for negative or low-positive antibody in 165 children who started ART 6 months of age (combining round 1 and 2). Note: excluding those not suppressed at measurement of antibody (N=5)

Characteristic	All 6 months at ART start (n = 165)	Undetectable or low Ab (n = 32)	Positive Ab (n = 133)	P-value
Sex, N (%)				
Male	74 (44.9)	13 (40.6)	61 (45.9)	0.593
Female	91 (55.1)	19 (59.4)	72 (54.1)	
Age when tested for AB (years)				
Mean $\pm$ SD	4.49 $\pm$ 0.6	4.43 $\pm$ 0.6	4.51 $\pm$ 0.6	0.522
Min, Max	3.52, 6.04	3.52, 5.91	3.52, 6.04	
Time on ART until AB testing (years)				
Mean $\pm$ SD	4.16 $\pm$ 0.6	4.19 $\pm$ 0.6	4.15 $\pm$ 0.6	0.784
Min, Max	3.01, 5.72	3.34, 5.72	3.01, 5.67	
CD4 percentage at time of AB testing				
Mean $\pm$ SD	35.6 $\pm$ 7.6	37.9 $\pm$ 7.4	35.1 $\pm$ 7.7	0.068
Min, Max	17.5, 56.9	18.4, 54.1	17.5, 56.9	
WAZ at time of AB testing				
Mean $\pm$ SD	-0.84 $\pm$ 0.9	-1.02 $\pm$ 0.9	-0.80 $\pm$ 0.9	0.218
Min, Max	-3.04, 1.35	-2.57, 1.22	-3.04, 1.35	
Regimen at time of AB test, N (%)				
LPV/r-based	69 (41.8)	12 (37.5)	57 (42.9)	0.582
EFV-based	93 (56.4)	19 (59.4)	74 (55.6)	
Other	3 (1.8)	1 (3.1)	2 (1.5)	
Highest known HIV RNA (copies/mL) after suppression initially, N (%)				
Always 50	84 (50.9)	21 (65.6)	63 (47.4)	0.027
51–1000	58 (35.2)	11 (34.4)	47 (35.3)	
>1000	23 (13.9)	0 (0.0)	23 (17.3)	
Birthweight (grams), Mean $\pm$ SD	2656 $\pm$ 859	2735 $\pm$ 796	2638 $\pm$ 875	0.567
Pre-treatment WAZ, Mean $\pm$ SD	-2.19 $\pm$ 1.6	-2.29 $\pm$ 1.7	-2.17 $\pm$ 1.6	0.817
Pre-treatment HIV RNA (copies/mL), N (%)				
<100,000	17 (11.9)	3 (11.1)	14 (12.1)	0.973
100,000–750,000	40 (28.0)	8 (29.6)	32 (27.6)	
750,000	86 (60.1)	16 (59.3)	70 (60.3)	
Pre-treatment CD4 percentage, Mean $\pm$ SD	24.7 $\pm$ 10.6	31.0 $\pm$ 13.4	23.1 $\pm$ 9.2	<0.001
Pre-treatment CD4 percentage, N (%)				

Characteristic	All 6 months at ART start (n = 165)	Undetectable or low Ab (n = 32)	Positive Ab (n = 133)	P-value
<10	9 (5.7)	1 (3.1)	8 (6.4)	0.010
10–14.9	20 (12.7)	2 (6.3)	18 (14.4)	
15–19.9	29 (18.5)	4 (12.5)	25 (20.0)	
20–24.9	25 (15.9)	1 (3.1)	24 (19.2)	
≥25	74 (47.1)	24 (75.0)	50 (40.0)	
Pre-treatment CD4 count, Mean ± SD	1413 ± 822	1529 ± 802	1384 ± 828	0.379

Note: ART – Antiretroviral therapy (ART); AB = HIV antibody; IQR = Interquartile range (25th percentile, 75th percentile); SD = standard deviation; WAZ = weight-for-age Z-score. Numbers do not always add up to totals due to missing data.