Routine Antibody Tests have No Place in Determining HIV-Status after Early ART: Evidence from CHER

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BACKGROUND

HIV-1-infected infants starting early ART may become HIV-antibody seronegative following decay of passively acquired maternal antibody and suppression of viral replication. As current ART guidelines recommend immediate ART for all HIV-infected children under 2 years[1], the frequency of seronegative status following early ART raises numerous questions about clinical management of young children[2]. As the possibility of functional cure with very early ART increases, it is important that the frequency and management implications of seronegative status in HIV-infected children receiving early ART are understood.

MATERIALS AND METHODS

The randomized Children with HIV Early Antiretroviral Therapy (CHER) trial compared early limited with deferred ART (ART-Def) in HIV-infected infants <12 weeks of age with baseline CD4 ≥25%[3, 4] (Figure 1). ART was limited to 40 or 96 weeks in the early treatment arms, ART-40W and ART-96W, respectively. HIV infection was diagnosed by HIV DNA PCR and confirmed with a RNA viral load >1000 copies/ml.

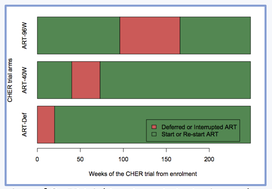

Figure 1: Overview of CHER trial treatment strategies and median age and duration on ART within arms. Median time to ART initiation in the deferred arm was 20 weeks (IQR 16-25). Median duration of ART-interruption for ART-40W was 33 (26-45) and 70 (35-109) weeks in ART-96W.

Here we compare early ART until 96 weeks (ART-96W) with ART-Def. Median age at ART start was 23 weeks (IQR 18-32) for ART-Def versus 7 weeks (IQR 7-8) for ART-96W. HIV-1 antibody was measured from the stored plasma of 75/125 ART-Def and 109/126 ART-96W children at 84 weeks of the CHER trial, which equals a median age of 91.4 weeks (IQR 90.6 – 93.4).

Three techniques were utilized to measure HIV-1 antibody in these samples: (1) 4th generation microparticle enzyme immunoassay HIV antigen/antibody combination (Abbott AXSYM system); (2) HIV-1/2 qualitative immunochromographic rapid antibody test (Alere Determine) assessed by an independent, blinded clinician; (3) A sensitive in-house ELISA to quantify anti-gp120 IgG and total IgG (NHLS AIDS Virus Laboratory, Johannesburg). Samples were measured against a 16-point standard curve of clade C gp120 monoclonal antibody or IgG from human serum. Confirmed positive and negative controls were included in each ELISA plate.

RESULTS

There were no significant differences between the groups ART-Def and ART-96W in gender, ethnicity, CD4 count/percentage or viral load at enrolment or time of HIV-1 antibody analysis (Table 1). As per trial design, there is a significant difference between age at ART initiation and children from ART-Def initiated ART according to clinical/CD4 indications compared to ART-96W who commenced ART at enrolment. At time of HIV-antibody analysis no child was on second line ART.

	ART-Def	ART-96W	
	n (%)		P-value
No. samples	75 (41)	109 (59)	NA
Male	33 (44)	48 (44)	0.99
Black	74 (99)	105 (96)	0.29
No. reached trial primary outcome	18 (24)§	16 (15)§	0.12
	Median [IQR]		P-value
CD4 at enrolment:	2,039	2,067	0.7
- Absolute count (cells/ul)	[1,587 – 2,861]	[1,520 -2,743]	
- Percentage	34 [28 - 41]	35 [30 - 39]	0.7
CD4 at antibody analysis	1,623	1,641	0.93
- Abs count (cells/ul)	[1,330 – 2,074]	[1,247 – 2,065]	
- Percentage	34.2 [31 – 40]	36.9 [31.7 – 42.1]	0.25
Viral load at enrolment	750,001	750,001	0.76
	[478,500 – 750,001]	[535,000 – 750,001]	
Viral load at antibody analysis	399	399	0.2
(84 wks of trial)	[399 - 399]	[399 - 399]	
Age of starting ART	0.44	0.14	<0.0001*
(decimal age in yrs)	[0.35 – 0.6]	[0.13 – 0.16]	

Table 1: Characteristics of children whose plasma were analyzed for HIV-antibody. §Reasons for reaching primary endpoint for ART-Def were: 3 Clinical, 7 Immunological, 8 Virological; and for ART-96W were: 2 Clinical, 13 Immunological, 1 Death. *As per CHER design.

Children in ART-Def had significantly more antibody than ART-96W in all 3 tests utilized by this study. 90% of children from ART-Def had a positive automated serology (4th generation microparticle enzyme immunoassay antigen/antibody combination) versus 54% seropositive in ART-96W (p<0.0001). As per Table 2, positive results are routinely divided into "reactive" and "weakly reactive" in diagnostic laboratories, and while accounting for this subdivision the difference remains equally significant. These results were also reflected in the HIV-1/2 qualitative immunochromographic rapid antibody test (88% versus 47%, p<0.0001).

RESULTS

	ART-Def n (%)		ART-96W n (%)		P-value
	Serology	Rapid	Serology	Rapid	
Reactive	62 (83)	64 (85)	38 (35)	41 (41)	<0.0001
Weakly reactive	5 (7)	2 (3)	20 (19)	6 (6)	
Non-reactive	8 (10)	8 (11)	49 (46)	54 (53)	
Insufficient	0	1	2	8	
Sensitivity	**89.3%**	**89.1%**	**54%**	**56.5%**	

Table 2: Comparison of 4th generation microparticle enzyme immunoassay HIV antigen/antibody combination ("Serology") and HIV-1/2 qualitative immunochromographic rapid antibody test ("Rapid") between ART-Def vs ART-96W. T-test for difference between CHER arms is <0.0001 for both routine antibody test methods.

Finally, there was also a significant difference with the quantitative anti-gp120 IgG ELISA (ART-Def median 6,870 µg/µl (IQR 1,706–53,645) versus ART-96W 230 µg/µl (IQR 133–13,129) p=0.04) (Figure 2). Anti-gp120 IgG quantity and serology results were strongly associated (anova p-value <0.0001). Levels of total IgG were similar in both groups (median 25,530 µg/µl (IQR 15,568-40,391) versus 25,993 µg/µl (IQR 17,779-44,241) p=0.28) indicating that this effect was specific to HIV responses.

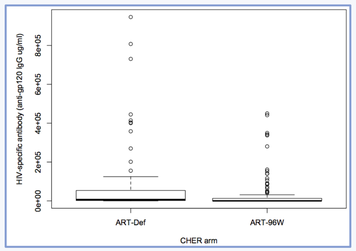

Figure 2: Box-whisker plot to illustrate the range of HIV-specific antibody (anti-gp120 IgG µg/µl) in two arms, ART-Def and ART-96W, of the CHER trial (p=0.04).

All children had detectable anti-gp120 IgG antibody by ELISA and none had an optic density of anti-gp120 lower than the HIV-uninfected controls. 8 (10%) from ART-Def and 49 (46%) from ART-96W were seronegative using automated serology. In ART-Def, there was no difference between the automated and rapid test, and although within trial arms there was no overall statistical difference between the two tests (T-test: 0.84 for ART-Def and 0.77 for ART-96W), in ART-96W 9 children with weakly positive automated results had negative rapid tests.

RESULTS

In ART-Def, older age at ART initiation was associated with increased anti-gp120 IgG (Spearman's rank correlation, p=0.002). By automated serology, starting ART between 12-24 weeks had a 13.7-fold higher odds of being seropositive at ~92 weeks of age, compared to starting ART aged 0-12 weeks (95% CI: 3.1 – 60.2, p=0.001); all 33 children starting ART <24 weeks of age were seropositive (Figure 3 and Table 3).

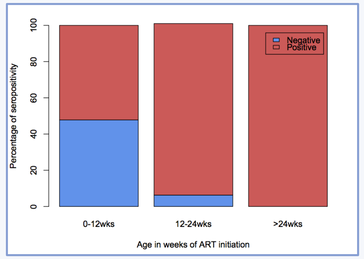

Figure 3: Relative frequency of HIV-1 antibody serology positivity at median 91.4 weeks of age with regard to age of ART initiation.

	Age in weeks at ART initiation, n (%)			
Serology	**0-12 wks**	**12-24 wks**	**>24 wks**	Total
Negative	55 (47.8)	2 (6.3)	0 (0)	57 (32)
Positive	60 (52.2)	30 (94.7)	33 (100)	123 (68)
Total	115	32	33	180

Table 3: Frequency of seropositivity relative to age at ART initiation.

Cumulative viral load from study enrolment until 84 weeks of the trial when the antibody analysis was performed was highly correlated with anti-gp120 IgG quantity (Spearman's rank correlation p<0.0001) (Figure 4).

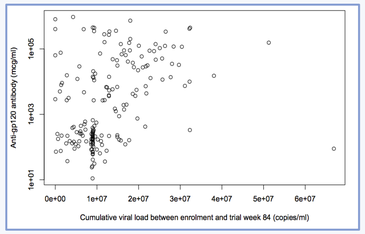

Figure 3: Relationship between cumulative viral load from enrolment until trial week 84 (the point at which the antibody analysis was performed). Spearman's rank correlation p<0.0001.

CONCLUSIONS

Almost half children starting ART within 12 weeks of birth and 6% of those starting at 12-24 weeks were seronegative by commercial serology or rapid tests at ~2 years of age. Therefore, routine antibody tests should not be used to confirm HIV status among children already diagnosed and started on ART at <6 months of age.

The association between HIV-1 viral load and anti-gp120 IgG magnitude suggests antibody quantity is directly related to HIV-1 antigen-stimulation, although whether persistence of anti-gp120 IgG represents slow decay or is a response to low levels of HIV replication, requires further study.

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