HIV antibodies by Western Blot in early-treated children living with HIV after a decade of treatment

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Louise Kuhn,[1] Mqondisi Tshabalala,[2] Renate Strehlau,[3] Avy Violari,[4] Faeezah Patel,[5] Afaaf Liberty,[4] Caroline T. Tiemessen[2]

1. Gertrude H. Sergievsky Center, Vagelos College of Physicians and Surgeons; and Department of Epidemiology, Mailman School of Public Health, Columbia University Irving Medical Center, New York, NY. 2. National Institutes for Communicable Diseases, and Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. 3. VIDA Nkanyezi Research Unit, Rahima Moosa Mother and Child Hospital, Department of Paediatrics and Child Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. 4. Perinatal HIV Research Unit, Chris Hani Baragwanath Hospital, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. 5. Wits RHI, Shandukani Research Centre, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

BACKGROUND

Some infants with perinatally-acquired HIV who begin antiretroviral therapy (ART) early in life may test HIV antibody negative in early childhood. Here we examined the frequency of testing antibody negative after almost a decade of ART and investigated other factors associated with this antibody profile.

METHODS

HIV-1 Western Blot assays were conducted on blood samples collected from 170 children living with HIV (CLWH) and 57 age-matched children without HIV (controls) at exit from an observational study undertaken at two sites in Johannesburg, South Africa. Western blots were coded as negative, indeterminate, or positive and reactivity to specific proteins were coded as negative, equivocal (weak), weak positive, or strong positive. CLWH had initiated ART in infancy and had been followed as part of studies for almost a decade. Detailed characteristics of the CLWH pre-ART and during ART had been collected, including all plasma viral load and CD4 measurements over time. Diagnostic HIV-1 nucleic acid amplification test (NAAT) was done at the same time point and cycle threshold values were used to estimate HIV-1 DNA levels.

RESULTS

In CLWH who had been on ART for about 9 years, 6.5% tested HIV-1 Western Blot negative and 30% tested indeterminate. Intensity of the specific proteins are shown below:

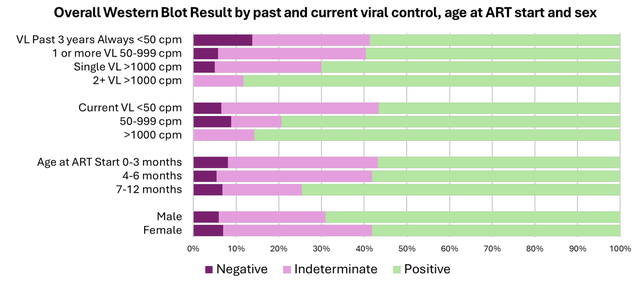


After almost a decade on ART, about a third of children who initiated ART in infancy have negative or indeterminate Western Blots. Negative/Indeterminate results are associated with younger age of ART start, more sustained plasma viral load suppression over time and lower levels of HIV DNA.

	Children living with HIV (n=170)	Children who are HIV uninfected (n=57)
Sex		
Male	84 (49.4)	32 (56.1)
Female	86 (50.6)	25 (43.9)
Age at Western Blot in years		
Median (p25, p75)	9.29 (8.57, 10.63)	9.07 (8.00, 9.82)
Viral load at Western Blot		
Undetectable <50 copies/ml	122 (71.8)	
51-999 copies/ml	34 (20.0)	
≥1000 copies/ml	14 (8.2)	
Viral load over prior 3 years		
Always <50 copies/ml	29 (17.1)	
At least one 51-999	104 (61.2)	
One >1000 copies/ml	20 (11.8)	
Two + >1000 copies/ml	17 (10.0)	
Current regimen		
LPV/r-based	47 (27.6)	
EFV-based	122 (71.8)	
Other	1 (0.6)	
Age at ART start		
<60 days	17 (10.0)	
61-90 days	20 (11.8)	
91-122 days	30 (17.7)	
123-180 days	44 (25.9)	
>181 days	59 (34.7)	
Age at ART start in days		
Median (p25, p75)	145 (96, 227)	
Pre-ART Viral load		
<100,000 copies/ml	13 (9.4)	
100,000-750,000	37 (26.8)	
>750,000 copies/ml	88 (63.8)	
Pre-ART CD4 %		
<25	92 (58.6)	
25-30	26 (16.6)	
>30	39 (24.8)	

COHORT CHARACTERISTICS

Median age of CLWH at Western Blot measurement was 9.3 years and most had an undetectable viral load at that time. Most had had no viral loads >1000 copies/ml over the prior 3 years, although always being undetectable was less common (~17%). Median age at ART start was just under 5 months of age.



FACTORS ASSOCIATED WITH WESTERN BLOTS

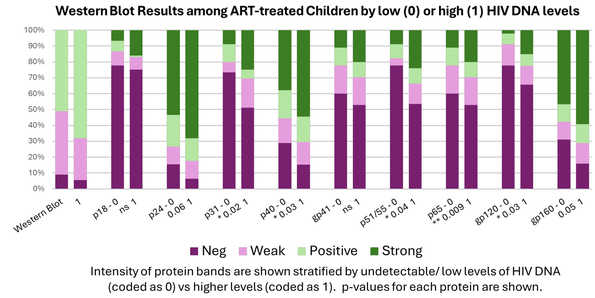
Always being undetectable <50 copies/ml in the prior 3 years was associated with a higher likelihood of testing WB negative (14%) than those with low level or a single VL >1000 copies (6% WB negative). No negative WB results were observed in those with more than one VL>1000 copies/ml in the prior 3 years.

Similarly, current viral load was associated WB results with 43% testing WB negative or indeterminate among those currently undetectable <50 copies/ml, 21% among those with VL 51-99 copies/ml and 14% among those with VL>1000 copies/ml.

Initiating ART under 6 months of age was associated with greater likelihood of testing WB negative or indeterminate.

There were no differences in the frequency of WB results by sex, pre-ART viral load, pre-ART CD4 percent or current regimen.

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Intensity of protein bands are shown stratified by undetectable/ low levels of HIV DNA (coded as 0) vs higher levels (coded as 1). p-values for each protein are shown.

ASSOCIATIONS WITH HIV-1 DNA LEVELS

We stratified the results of a diagnostic HIV-1 NAAT combining those testing NAAT negative with the top 20% of the highest cycle threshold values into a category of low levels of HIV-1 DNA (displayed as 0 above). Those with cycle threshold values lower than this threshold were in the high level category (displayed as 1 above).

The overall WB result trended towards being more likely to be negative or indeterminate in those with estimated low levels of HIV-1 DNA than in those with higher levels (p=0.06). Several specific proteins detected on WB had significant associations with levels of HIV-1 DNA including p31, p40, p51/55, p65 and gp120.

CONCLUSIONS

HIV-1 Western Blots are a relatively simple means of establishing the extent of past viral load control on ART. Negative and indeterminate results are fairly common in well-controlled cohorts of CLWH who started ART in infancy. Negative and indeterminate results are associated with younger age of ART start, more sustained plasma viral load suppression over time and lower levels of HIV DNA. Although an informative marker, caution is required as negative results do not indicate that HIV has been cleared.

PLAIN LANGUAGE SUMMARY

Western Blots provide an indirect history of the extent of viral control on ART over prior years in children who started ART in infancy. Negative or indeterminate Western Blot results, in turn, correlate with lower levels of viral DNA - a marker of a smaller viral reservoir.