Reactivity of routine HIV antibody tests in children with perinatally-acquired HIV-1 in England: cross sectional analysis

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ABSTRACT (60 words)

We assessed HIV antibody prevalence in children with perinatally-acquired HIV (PaHIV) in England. 18%(10/55) of those starting combination antiretroviral therapy (cART) <6 months of age were seronegative, and had lower viral load at diagnosis and cART start, and fewer viral rebounds, than 45/55 seropositives. Implications for patient selection for HIV cure research, and interpretation of routine antibody testing, are discussed.

INTRODUCTION

Children with perinatally-acquired HIV (PaHIV), both untreated and treated with combination antiretroviral therapy (cART), typically lose maternal transplacentally acquired antibody in the first 18 months of life and develop their own HIV antibody responses.[1] However in very early treated children with persistent viral suppression, the development of HIV specific antibodies may not occur, a phenomenon termed seroreversion or seronegativity.[2] Follow up of such children has been commonly only until age two years, and sample sizes have been relatively small.[3-6]

Results from the largest early treatment cohort to date, the Children with HIV Early Antiretroviral Therapy (CHER) trial, showed that 46% of 107 children commencing cART <12 weeks of age were seronegative at two years.[7] Only a few studies have investigated antibody status in older children. In Germany, 30% of 37 children commencing early cART (<5 months of age) and maintaining viral suppression were seronegative,[8] although only 5 (14%) remained antibody negative at a mean age of 6.6 years. In a cross-sectional study of 144 PaHIV children in the USA, median age 14.3 years, 34 had achieved virological control by one year of age.[9] Of these 34, 14 had full data available, for whom cART was commenced at a median of 2.4 months of age, and five (36%) were seronegative at last follow-up. In comparison, of 53 achieving virological control by age 1-5 years, and 77 by age >5 years, 2 (4%) and 0 (0%) were seronegative. A third study from Spain followed 14 PaHIV children who initiated cART <12 weeks after birth and 9 who initiated between week 12 and 1 year, all of whom maintained viral suppression for one year. At a median age of 8 years, 7/14 (50%) and 1/9 (11%) respectively had a negative or indeterminant HIV antibody result.[10] Long term immunological, virological and clinical consequences of seronegativity are not known but may be important, and HIV seronegative children may be ideal candidates for future HIV cure research.[11]

The aims of this study were to estimate the prevalence of HIV seronegativity in children with PaHIV in 5 paediatric HIV clinics in England, assess associated factors, and determine HIV-related outcomes, to inform future practice.

METHODS

Children with HIV in the UK/Ireland are initially reported to the National Study of HIV in Pregnancy and Childhood, and followed up by Collaborative HIV Paediatric Study (CHIPS), a national cohort of all children with diagnosed HIV.[12] HIV antibody status was collected by CHIPS since 2014, and five paediatric HIV clinics in South/Central England which were performing HIV antibody testing (4th generation Abbott or BioMérieux antigen/antibody test) as part of routine care at this time provided retrospective data. Antibody testing was initially introduced to confirm HIV diagnosis in children who had transferred from other clinics (which had undertaken original HIV diagnostic testing) and were clinically well with normal CD4s and long-term undetectable viral load. It was then extended to all children attending for HIV care at these clinics. Dates of birth and antibody results of children with HIV antibody tests >2 years of age at the 5 clinics were linked to CHIPS.

CHIPS data on socio-demographics and HIV-related parameters were analysed to ascertain predictors for HIV seronegativity as well as HIV-related outcomes. CHIPS has ethical approval from the London Multicentre Research Ethics Committee. All children with a negative HIV antibody test commenced cART <6 months of age and were compared to all seropositive children starting cART <6 months of age at the same 5 centres. Descriptive statistics summarised patient characteristics, and Fisher's exact and Wilcoxon Rank Sum tests were used to compare percentages and medians respectively. All analyses were undertaken using Stata 14 (College Station, Texas).

RESULTS

A total of 398 children with HIV were followed up by the five clinics, comprising 50% of the 794 children in active CHIPS follow-up in England to April 2016. Of these 398 children, 288 (72%) had a documented HIV antibody test result >2 years of age. Eleven (4%) were seronegative, of whom 10 had full CHIPS data available, and all ten started cART <6 months of age. 277 were seropositive of whom 45 started cART at <6 months of age.

Overall prevalence of seronegativity was 18% (10/55) in children treated <6 months of age. Median age at diagnosis of HIV and last follow-up was 0.2 years and around 10 years respectively (Table 1). Around half in each group were male, most were black African, and half were born before 2006. Of the ten seronegative patients, five (50%) were followed from birth, and five (50%) presented with symptoms in the first 6 months of life, compared to eight (18%) and 30 (67%) of seropositives (p=0.064). Three of the 10 seronegative patients were diagnosed with HIV on the day they were born, of whom two (20%) received prophylaxis for prevention of mother to child transmission (PMTCT) from birth and one started ART a month later, whilst only 4 of 45 seropositive patients (9%) received PMTCT prophylaxis from birth. Median age at cART start was similar at around 0.3 years of age.

Median CD4 percentage and count at diagnosis, and median CD4 count at last follow-up, were similar between the two groups. However median VL at diagnosis in the seronegatives was considerably lower than the seropositives (51,000c/mL vs 500,000c/mL, p=0.03), and median VL at cART start was also lower in the seronegatives (111,651c/mL vs 500,000c/mL, p=0.049). However after six and 12 months of cART, and at last follow-up, there were no differences between the groups in the proportion with VL ≤50c/ml and/or under the limit of detection. Similarly there were no differences between these groups in the median cumulative years suppressed or CDC stage C at last follow-up. However prevalence of viral rebound ≥400c/mL following initial virological suppression after cART initiation was 11% (1/9) in seronegative children compared to 69% (29/42) in seropositive children (p=0.002) with complete viral load data. The one seronegative patient with viral rebound 8 years after starting cART had viral loads of 670c/mL and then 3,232c/mL 14 days apart, subsequently reverting back to suppression <400c/mL, and remained seronegative >2 years later. The other eight with complete viral load data remained <400c/mL following initial suppression.

DISCUSSION

This study reports characteristics of some of the oldest children to date in Europe with PaHIV and HIV seronegativity. Overall prevalence of seronegativity was 4% in PaHIV children >2 years of age, and 18% in those who commenced cART <6 months of age and followed up at a median age of 9.1 years. HIV antibody seronegativity was only seen in children who commenced cART <6 months of age and these children had significantly lower viral loads at diagnosis and cART start, and fewer episodes of viral rebound during follow-up than seropositive children. Prevalence in our study was broadly comparable to two other cohorts of older children from Germany and the USA (14%, 36% respectively).[8, 9] In the CHER cohort almost half of early treated infants were seronegative at 2 years of age but longer term analyses at older ages are awaited.[7]

Antibody status in PaHIV may be an indication of immunological function. Early initiation of cART and virological control soon after infection, in both adults and infants, limits the size of the latent HIV reservoir.[10, 13, 14] and it is presumed that lower levels of HIV antigenaemia abrogates the specific antibody response. In our cohort seronegativity was associated with lower median HIV viral load at diagnosis and at cART initiation which would be consistent with other studies that suggest that seronegativity is associated with lower proviral DNA.[9] The association of early treatment with lower proviral DNA was also reported in the CHERUB-YC cohort, 40% (4/10) of whom were seronegative at a median age of 9.9 years.[15] Whether seronegativity and a low viral reservoir could be used to aid selection of paediatric patients for future interventional trials for cure strategies remains unclear, and is being explored within the EPIICAL collaboration.[11]

Overall long term viral suppression and the possible "surrogate marker" of suppression i.e. antibody seronegativity, may well have long term immune benefits[11] but may inadvertently cause problems in interpretation of subsequent confirmatory HIV antibody tests. In high income settings this could be in Emergency departments, sexual health clinics, blood donation centres or following transition to adult services. Careful communication to both the young person and other medical professionals that the young person does still have HIV is required.

This study has limitations. Firstly, only five clinics providing paediatric HIV care were included. However selection bias of clinics is unlikely to be a concern as seronegative patients are also found in other areas of the UK/Ireland (K Fidler, personal communication). Secondly HIV reservoir and HLA allele data were not available as this was primarily an antibody prevalence study. A previous study suggested a possible genetic predisposition to seronegativity dependent upon HLA class 2 alleles, although numbers were small.[8] Finally our estimated prevalence may have been biased by only 288/398 children at the 5 centres had an antibody test; for the rest these were either not performed as the child was less than 2 years of age, or were not done or not recorded.

With international guidelines recommending cART initiation in all children living with HIV, irrespective of CD4 count, and an emphasis on early treatment, it is likely that the number and proportion of children with HIV who have negative HIV antibody test results will increase in the future. Further long-term follow-up of these children can help elicit how seronegativity is associated with viral dynamics. Patients and medical staff need to be counselled to understand that a negative HIV antibody test in this situation does not mean lack of HIV infection and is not an indication to cease treatment.

Author Contributions

KF, EL, and NK conceived the idea for the study, and KF led its design, coordination and manuscript write up. AJ analysed the CHIPS data and was a major contributor to manuscript preparation. AP was involved in initial analysis of seronegative patients. All authors contributed data to the study via CHIPS and/or helped with manuscript preparation. All authors read and approved the final manuscript.

Conflict of interest

None declared.

Ethics

The CHIPS study has ethical approval from the London Multicentre Research Ethics Committee.

Table 1: Characteristics of 55 seronegative and seropositive children with perinatal HIV starting cART <6 months of age

Characteristic		Total		Seronegative (n=10)		Seropositive (n=45)		P value
				n % or median [IQR]				
Region of clinic	Outside London	13	24%	4	40%	9	20%	
	London	42	76%	6	60%	36	80%	0.17
Place of birth	UK/Ireland	52	95%	10	100%	42	93%	
	Abroad/not known	3	5%	0	0%	3	7%	1.00
Sex	Male	24	44%	5	50%	19	42%	
	Female	31	56%	5	50%	26	58%	0.73
Ethnicity	White	2	4%	0	0%	2	5%	
	Black African	41	76%	9	90%	32	73%	
	Other	11	20%	1	10%	10	23%	0.78
Year of birth	Up to 2005	33	60%	5	50%	28	62%	
	2006 onwards	22	40%	5	50%	17	38%	0.50
When/ how child identified	Prospectively from birth	13	24%	5	50%	8	18%	
	After birth, asymptomatic	7	13%	0	0%	7	16%	
	After birth, symptomatic	35	64%	5	50%	30	67%	0.064
ART prophylaxis from birth		6	11%	2	20%	4	9%	0.306
Age at diagnosis		0.2	[0.1,0.3]	0.2	[0.0, 0.3]	0.2	[0.1, 0.3]	0.38
Age started cART		0.3	[0.2, 0.4]	0.3	[0.1,0.3]	0.3	[0.2,0.4]	0.13
Age at last follow-up		10.0	[6.7, 13.8]	9.1	[4.7,11.2]	10.2	[7.0,14.3]	0.13
CD4% at diagnosis				31	[17, 50]	27	{14, 42]	0.52
CD4 at diagnosis		1164	[287, 2152]	702	[280,2033]	1290	[388,2152]	0.69
CD4 at last follow-up		1047	[769, 1441]	1095	[788,2013]	1047	[769,1420]	0.56
Viral load at diagnosis		500000	[27676,799920]	51,000	[1147,500000]	500000	[43756,880000]	0.03
Viral load at start of cART		438405	[40378,774960]	111651	[18900,338637]	500000	[60778,925000]	0.049
Viral load at 6 months of cART	≤50 or <lower limit	22	43%	4	44%	18	43%	
	>50 or >lower limit	29	57%	5	56%	24	57%	1.00
Viral load at 12 months of cART	≤50 or <lower limit	30	61%	6	67%	24	60%	
	>50 or >lower limit	19	39%	3	33%	16	40%	1.00

Viral load at last follow-up	≤50 or <lower limit	48	87%	10	100%	38	84%	
	>50 or >lower limit	7	13%	0	0%	7	16%	0.22
Median days from cART start to VL ≤50 or <lower limit		186	[95, 436]	186	[65, 218]	184	[97, 507]	
Cumulative years suppressed <400 c/mL		7.0	[4.5, 10.3]	8.5	[4.2,10.9]	6.9	[5.0,10.2]	0.81
Viral rebound ≥400c/mL after initial suppression <400c/ml		30	59%	1	11%	29	69%	0.002
CDC stage at presentation	N/A/B	29	54%	4	44%	25	56%	
	C	25	46%	5	56%	20	44%	0.72
CDC stage at last follow-up	N/A/B	21	38%	4	40%	17	38%	
	C	34	62%	6	60%	28	62%	1.00

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