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Serological response to 13-valent pneumococcal conjugate vaccine in children and adolescents with perinatally acquired HIV infection

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Background: Children with perinatally acquired HIV (paHIV) remain at an increased risk of pneumococcal infection despite highly active antiretroviral therapy (HAART). Beyond infancy, responses to pneumococcal conjugate vaccine (PCV) remain under-investigated. There are currently no published data on serological response to 13-valent PCV (PCV13) in the HIV-infected populations.

Methods: We measured pneumococcal serotype-specific IgG in 48 paHIV-infected child patients (CP), 27 young adult healthy controls (AHC) and 30 child healthy controls (CHC). Opsonophagocytic assay (OPA) titres for three PCV13-exclusive serotypes were measured in a subset of children. Serotype-specific IgG was repeated 1 and 6 months following PCV13 vaccination of CP and AHC groups. OPA titres for four serotypes were measured at the 1-month time-point.

Results: The majority of CP, CHC and AHC had serotype-specific IgG above 0.35 μg/ml at baseline, although OPA activity was undetectable for two of the three serotypes studied. Baseline IgG concentrations were significantly lower in CP than AHC for a proportion of serotypes and were strongly predictive of responses to vaccine. After adjusting for baseline, postvaccination IgG concentrations were comparable, although responses to some serotypes were impaired for CP. OPA correlated well with IgG after vaccination. Detectable HIV viral load was associated with significantly lower IgG concentration and OPA titre.

Conclusion: Children with paHIV mount a robust serological response to PCV13 for most but not all vaccine serotypes. Viral load suppression with HAART and higher baseline IgG concentration are associated with higher postvaccination antibody levels. This has implications for HAART treatment and vaccination practices.

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Introduction

Children with perinatally acquired HIV (paHIV) receiving HAART continue to have an increased risk of invasive pneumococcal disease (IPD) [1]. Pneumococcal conjugate vaccines (PCVs) have proven efficacy when administered to HIV-infected infants [2,3] and adults [4], and should therefore be efficacious in older children and adolescents with paHIV. However, there are no published data to date on 13-valent PCV (PCV13) in any HIV-infected age group and few studies of licensed PCV in older HIV-infected children [5–12].

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Studies of lower-valency PCVs (ranging from 4 to 9 serotypes) in HIV-infected individuals including infants, older children and adults have generally shown that immunogenicity is impaired in comparison to healthy controls (reviewed in [13]). The response to pneumococcal vaccination is likely to be different in older children with paHIV compared to HIV-infected infants or adults. In infants with paHIV, pneumococcal conjugate vaccine will mainly induce a primary response, on the background of an immature immune system already burdened with high viral loads, immune activation and $CD4^+$ T-cell depletion [14]. In contrast, adults with horizontally acquired HIV have already encountered pneumococci and established a degree of immunity to many of the pathogenic serotypes by the time of acquisition of HIV [15]. Pneumococcal vaccination response in this context will be determined in part by the specific impact of HIV on the fully developed and pneumococcal antigen-experienced immune system. Although an older child with paHIV has survived infancy, their immune system has developed (or is still developing) under the influence of HIV, with or without the benefit of HAART. They are likely to have some degree of pneumococcal immunity through natural exposure, but this will have been modified by alterations of both the innate and acquired immune system related to HIV infection. Abnormalities of immune phenotype found to be characteristic of this patient group are likely to impact on the responses to PCV.

Debate as to whether PCV13 should be recommended for older children in risk groups such as paHIV is ongoing [16–19]. There is little evidence to guide practice with respect to timing of immunization relative to HAART and dosing schedules in older children. Immunogenicity data make an essential contribution to evidence-based vaccination guidelines and policy for at-risk groups. In order to provide immunogenicity data while investigating factors affecting vaccine response, we studied both quantitative and qualitative responses to PCV13 and analysed them according to treatment history, viral load and other clinical parameters in a cohort of children and adolescents with paHIV.

Methods

Study population and design

The study took place in the HIV Family Clinic at Imperial College Healthcare National Health Service (NHS) Trust, St Mary's Hospital, London, UK, between August 2009 and October 2012. Children with paHIV and healthy HIV-uninfected children aged between 2 months and 18 years, and young HIV-uninfected adults aged between 18 and 30 years were recruited from the HIV Family Clinic, routine preop assessment clinics/general practitioner (GP) phlebotomy clinics and through advertisement in Imperial College London, respectively. Exclusion criteria included other causes of immunosuppression, previous reaction to PCV or its components, current febrile illness and pregnancy. Ethical approval was obtained from the Riverside Research Ethics Committee, Charing Cross Hospital, London (Reference: 09/H0706/23). Parents of children included in the study and adult controls provided written informed consent. For child cohorts, background demographic and clinical information were obtained from clinical notes and GP, hospital pathology and Personal Child Health records. For adult controls information was obtained from interview and/or GP records.

Perinatally acquired HIV-infected child patients had blood sampled at baseline for lymphocyte subsets (LSS), plasma HIV-RNA viral load and serological studies. They then received one dose of PCV13 [2.2 μg pneumococcal polysaccharides 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 23F and 4.4 μg 6B, conjugated to CRM197 carrier protein, adsorbed on aluminium phosphate (0.125 mg aluminium)] (Pfizer, UK; batch number: E27415), administered as an intramuscular injection into the anterolateral thigh for infants and the deltoid muscle for older children and adults. Vaccinees and their families were asked to contact the research team should they suffer adverse events. History of adverse events was sought at each contact. Blood samples were obtained at 1 month and again at 6–9 months after immunization. Child healthy controls (CHC) had blood sampled once only for LSS and serological studies. For young adult healthy controls (AHC), blood sampling and vaccination was as for the CP cohort. For ethical and logistic reasons, only CP and AHC were vaccinated with PCV13. The CHC group was included to enable an age-related assessment of baseline immunity and in order to validate the suitability of AHC as a control group.

Laboratory methods

HIV viral load was measured using branched-chain DNA assay (Siemens Healthcare, Sudbury, UK) with a lower limit of detection of 50 copies/ml, and quantitation of LSS was undertaken by staining whole blood (EDTA) with monoclonal antibodies [CD45-FITC, CD4-RD1, CD8-ECD, CD3-PC5, CD56-RD1, CD19-ECD, CD16-PE (Beckman Coulter Inc., High Wycombe, UK)] and evaluated using FC500 flow cytometer (Beckman Coulter Inc.) via NHS laboratory services. Serological assays were performed in the WHO reference laboratory for pneumococcal serology at the Institute of Child Health, London, UK. Pneumococcal capsular polysaccharide-specific IgG for all PCV13 strains was measured by ELISA after absorption with cell wall and 22F polysaccharide. Opsonophagocytic assay (OPA) was performed according to methods of Romero-Steiner using the promyelocytic HL60 cell line. Detailed protocols for both methods are available at http://www.vaccine.uab.edu/.

Statistical analysis

For baseline characteristics and LSS, chi-square and Fisher's exact tests were used to compare proportions. Mann–Whitney U test was used to compare age and baseline LSS between the child and adult groups. To compare LSS between child groups, data were log-transformed and compared using linear regression analysis to allow adjustment for age and to assess age × group interactions. For serological data, chi-square and Fisher's exact tests were used to compare baseline proportions above a cut-off between groups. A cut-off of 0.35 μg/ml was chosen as this is an accepted correlate of protection in PCV immunogenicity studies in infants [20]. A more conservative cut-off of 1 μg/ml, potentially more relevant to long-term protection and allowing comparison with other published data in children with paHIV [11], was included in the analysis. One-way analysis of variance (ANOVA) with Bonferroni correction was used to compare log-transformed antibody concentrations and OPA titres at baseline between groups. Chi-square was used to compare proportions above a cut-off between groups at each time-point after immunization. McNemar's test was used to detect changes in proportion from baseline within groups. Repeated-measures ANOVA and analysis of covariance were used to compare log-transformed antibody concentrations between and within group. Post-hoc analyses for simple effects were then performed as appropriate. Linear regression was used to assess for associations between clinical parameters and log-transformed serology at baseline and 1 month after immunization. Factors assessed included: HIV viral load above 50 copies/ml (yes/no), HAART commenced in the first year of life (yes/no), HAART commenced in the first 2 years of life (yes/no), received PCV7 (yes/no), received pneumococcal polysaccharide vaccine (PPV) (yes/no), proportion of life with undetectable viral load and nadir $CD4^+$ percentage. A value of P less than 0.05 was considered statistically significant while acknowledging that a lower cut-off might be considered more appropriate in the context of multiple comparisons. Data were analysed using Stata IC version 12.1 for Mac (StataCorp, College Station, Texas, USA) and Prism 5 for Mac OS X (GraphPad, La Jolla, California, USA).

Results

Recruitment and baseline characteristics

An overview of study numbers and recruitment are shown in Fig. 1. Baseline characteristics are summarized in Table 1. In line with the study design and target populations, there were significant expected differences in ethnicity and country of birth (48% of CP were born in Africa). For those CP who had ever started HAART, median age at HAART initiation was 41 months [interquartile range (IQR) 11–91 months]. Sixty-five percent commenced HAART before 5 years of age. Median BMI centile for CP was 55% (IQR 25–91%). Sixty-three percent had a BMI centile greater than 50%. Twenty-three percent of CP had ever had an opportunistic infection. No AHC had received PCV or PPV, and no study participant had ever received PCV13. Prior to study entry, 20.1 and 4.2% of CP had received one or two doses of PPV, respectively (median time since last dose of PPV 46 months). Two and three doses of PCV7 were received

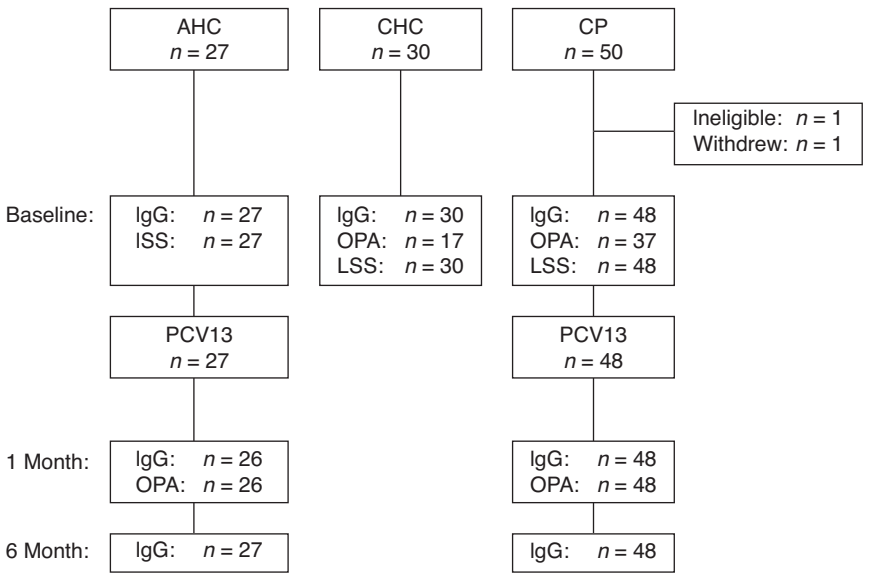

Fig. 1. Overview of study recruitment. One CP withdrew consent prior to blood sampling, one CP was found to be ineligible for study entry according to inclusion criteria, one AHC was excluded from 1-month serology analysis due to insufficient sample. AHC, adult healthy control; CHC, child healthy control; CP, child patient; IgG, pneumococcal serology; LSS, lymphocyte subsets; OPA, opsonophagocytic assay.

Table 1. Baseline characteristics for all study participants.

	CHC ($n=30$)		AHC ($n=26$)		CP ($n=48$)	
Sex						
Female	14	(46.7)	15	(57.7)	21	(43.8)
Male	16	(53.3)	11	(42.3)	27	(56.3)
Ethnicity						
White	4	(13.3)	19	(73.1)	2	(4.2)
Mixed	0	(0)	2	(7.7)	3	(6.3)
Asian	0	(0)	3	(11.5)	2	(4.17)
Black Caribbean	4	(13.3)	0	(0)	2	(4.17)
Black African	13	(43.3)[a]	0	(0)	38	(79.2)[b]
Other	9	(30)	2	(7.7)	1	(2.1)
Born in UK	29	(96.7)[a]	20	(74.1)	24	(50)[b]
Age/months	107.5	(19–195)	330[c]	(245–359)	154[d]	(12–209)
Previous PCV7	9	(30.0)	0	(0)	7	(14.6)
Previous PPV	0	(0)	0	(0)	12	(25.0)
Nadir CD4%	–	–	–	–	13	(0–48)
Prop	–	–	–	–	0.49	(0–0.98)
VL <50 copies/ml	–	–	–	–	37	(77.1)
Antiretroviral treated 1st year	–	–	–	–	7	(14.9)
Antiretroviral treated 1st 2 years	–	–	–	–	12	(25.3)
LSS						
Lymph cells/μl	2139	(1666–3414)	1807.5[e]	(1377–2115)	2349.5[f,g]	(1764–3308.5)
CD3 cells/μl	1575.5	(1166–2486)	1389[d,e]	(1094–1706)	1854.5[h]	(1390.5–2518)
CD3%	71.6	(66.6–78.3)	75.3	(70.1–79.1)	75.45	(68.9–79.6)
$CD4^+$ cells/μl	833.5	(647–1391)	794	(694–1061)	855[h]	(525.5–1118)
CD4%	43.1	(36.7–46.5)	44.6[e]	(39.8–50.6)	34.55[h]	(27.2–40.8)
$CD8^+$ cells/μl	529.5	(356–907)	497[e]	(383–557)	913.5[f,g]	(659–1074.5)
CD8%	23.8	(21.6–27.2)	26.3[e]	(22.2–28.9)	32.95[h]	(28.9–44.5)
CD19 cells/μl	446.5	(272–679)	257[d,e]	(153–326)	403[f]	(266–570.5)
CD19%	18.2	(14.5–24.2)	12[d,e]	(8.9–16.8)	17.3[f]	(12.96–21.4)
CD56 cells/μl	134.5	(82–205)	172.5[e]	(96–188)	119[h]	(72–184.5)
CD56%	5.4	(2.9–8.2)	9.4[d,e]	(6.5–12.1)	4.35	(2.8–5.6)

Sex, ethnicity, UK origin, HIV viral load status, treated in the first year of life, treated in the first 2 years of life, previous PCV7 and previous PPV are presented as number and percentage. Age, nadir CD4% and proportion of life undetectable are presented as median and range. Lymphocyte subsets are presented as median and inter-quartile range. Chi-square and Fisher's exact tests were used to compare proportions. Mann–Whitney *U* test was used to compare age and baseline LSS between the child and adult groups. To compare LSS between child groups, data were log-transformed and compared using linear regression analysis to allow adjustment for age and to assess age × group interactions. AHC, adult healthy control; CHC, child healthy control; CP, child patient; LSS, lymphocyte subsets; PCV7, 7-valent pneumococcal conjugate vaccine; PPV, pneumococcal polysaccharide vaccine; Prop, proportion of life spent with undetectable viral load; VL, HIV viral load.
[a]Proportion significantly different: CHC vs. AHC ($P<0.05$).
[b]Proportion significantly different: CP vs. CHC ($P<0.005$).
[c]Median significantly different from CP and CHC ($P<0.001$).
[d]Median significantly different from CHC ($P<0.05$).
[e]Median significantly different from CP ($P<0.05$).
[f]Significant independent age effect when comparing CHC vs. CP ($P<0.05$).
[g]Significant independent group effect when comparing CHC vs. CP ($P<0.05$).
[h]Significant age × group interaction when comparing CHC vs. CP ($P<0.05$).

by 3.6 and 8.9% of CP, respectively (median time since last dose of PCV7 13 months). In addition 6.7, 3.3 and 20% of CHC had received one, two or three doses of PCV7 respectively (median time since last dose of PCV7: 26 months).

Lymphocyte subsets

Results for each LSS are summarized in Table 1. Overall, these results confirmed significant age-specific differences in lymphocyte counts and proportions between predominately HAART-treated children with paHIV, healthy children and healthy young adults.

Baseline serology

Reverse cumulative distribution curves including baseline serum pneumococcal polysaccharide-specific IgG concentrations for AHC, CHC and CP are shown in Fig. 2. Geometric mean concentrations (GMCs) of IgG and proportion in each group above a cut-off of 0.35 and 1 μg/ml at baseline are reported in Table 2. Comparing CP with AHC, GMCs and proportions above 0.35 and 1 μg/ml were significantly lower in CP than in AHC for the four serotypes (7F, 9V, 18C and 23F). Both GMC and proportion above 0.35 μg/ml were significantly lower in CP than in AHC for serotype 14, whereas GMC and proportion above 0.35 μl were significantly lower in CP than in AHC for serotypes 19F and 6B, respectively. Comparing CP with CHC, GMC and proportions above 0.35 and 1 μg/ml were significantly lower in CP than in CHC for two serotypes (6B and 23F). Both GMC and proportion above 1 μg/ml were significantly lower in CP than in CHC for serotype 9V. Proportion above

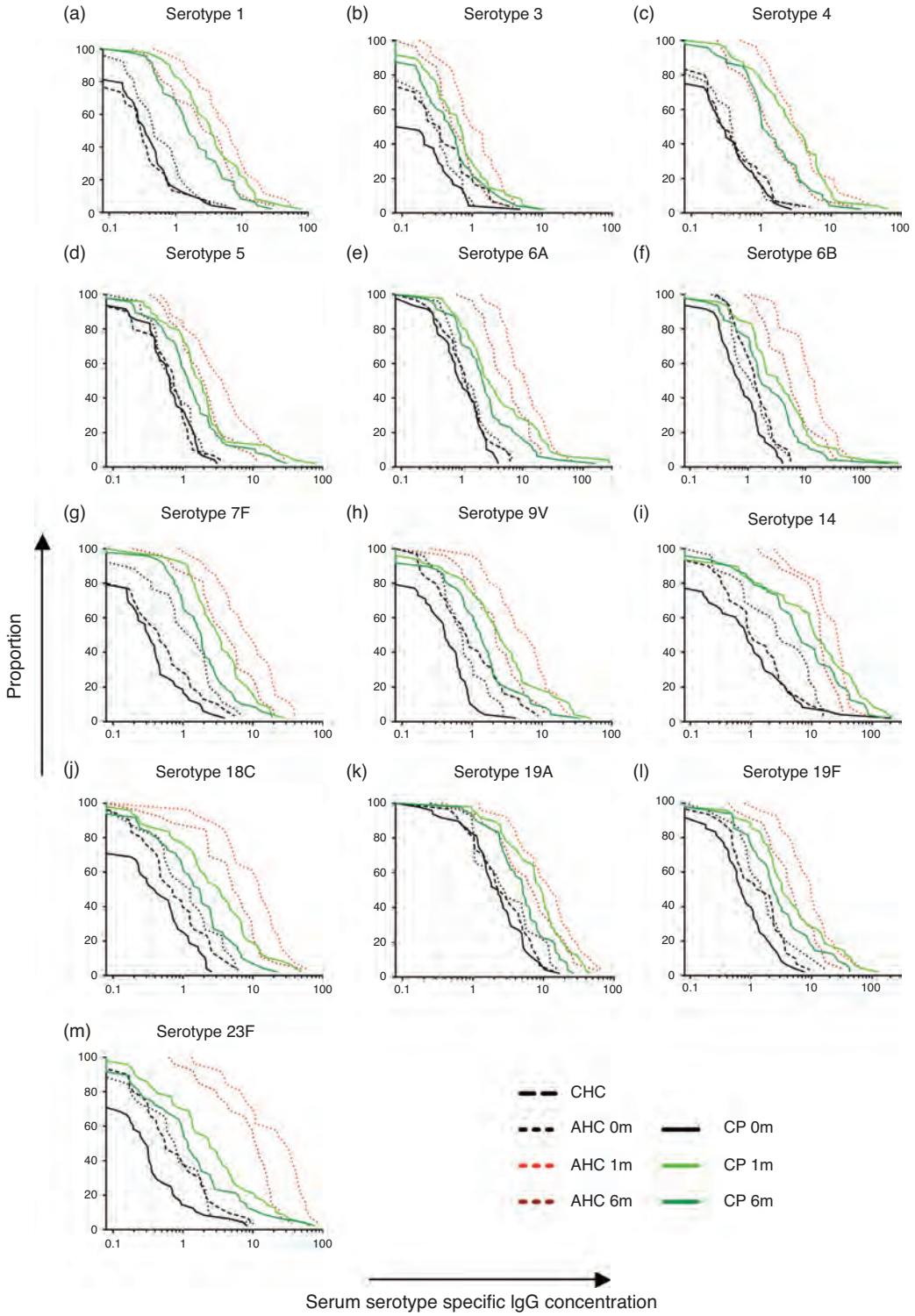

Fig. 2. (a–m) Reverse cumulative distribution curves showing the proportion (%) of individuals above increasing concentrations of serotype-specific antibody for healthy children, young healthy adults and children with perinatally acquired HIV. Baseline antibody concentration data are displayed in black. Post-immunization data for young healthy adults are shown in red and for HIV-infected children in green. Vertical grey lines indicate cut-off of 0.35 and 1 μg/ml. 0m, baseline; 1m/6m, 1 month/6 months after immunization; AHC, adult healthy control; CHC, child healthy control; CP, child patient; mcg, μg; S, serotype.

Table 2. Geometric mean concentration of pneumococcal polysaccharide serotype-specific IgG, proportion with concentration above 0.35 μg/ml and proportion with concentration above 1.0 μg/ml for each group at baseline and for vaccinated groups at 1 and 6 months after immunization.

ST	Time-point	GMC (μg/ml)			Proportion >0.35 μg/ml			Proportion >1.0 μg/ml		
		CHC	AHC	CP	CHC	AHC	CP	CHC	AHC	CP
1	0m	0.29	0.51	0.35	0.4	0.62	0.48	0.1	0.27	0.15
	1m	–	5.43	3.32	–	1.00[a]	0.98[a]	–	0.92[a]	0.81[a]
	6m	–	2.35	1.75	–	0.96[a]	0.94[a]	–	0.65[a]	0.67[a]
3	0m	0.30	0.25	0.18	0.4	0.38	0.27	0.16[b]	0.03	0.02
	1m	–	1.00	0.60	–	0.85[a]	0.65[a]	–	0.46[a]	0.29[a]
	6m	–	0.54	0.44	–	0.77[a]	0.58[a]	–	0.19	0.21[a]
4	0m	0.33	0.33	0.29	0.47	0.58	0.42	0.2	0.15	0.15
	1m	–	3.29	2.53	–	1.00[a]	0.92[a]	–	0.88[a]	0.75[a]
	6m	–	1.27	1.21	–	0.81[a]	0.83[a]	–	0.58[a]	0.52[a]
5	0m	0.56	0.66	0.58	0.77	0.85	0.77	0.27	0.35	0.31
	1m	–	3.22	1.76	–	1.00	0.88	–	0.81[a]	0.73[a]
	6m	–	1.74	1.23	–	0.96	0.85	–	0.73[a]	0.56[a]
6A	0m	1.03	1.21	0.82	0.83	0.96	0.79	0.57	0.54	0.48
	1m	–	10.54[c,d]	3.56	–	1.00	0.98[a]	–	1.00 [a,e]	0.81[a]
	6m	–	5.18[c,d]	2.20	–	1.00	0.92	–	0.96 [a,e]	0.71[a]
6B	0m	1.29[f]	1.08	0.68	0.97[b]	0.96[e]	0.73	0.63[b]	0.50	0.40
	1m	–	11.32[c,d]	3.31	–	1.00	0.94[a]	–	1.00 [a,e]	0.83[a]
	6m	–	5.12[c,d]	2.04	–	1.00	0.88[a]	–	0.96 [a,e]	0.75[a]
7F	0m	0.41[g]	0.91[c]	0.31	0.53	0.77[e]	0.46	0.27	0.50[e]	0.17
	1m	–	7.68[c]	3.01	–	1.00[a]	0.96[a]	–	0.96[a]	0.92[a]
	6m	–	3.45 c	1.66	–	0.96	0.94[a]	–	0.85[a]	0.65[a]
9V	0m	0.81[f]	0.67[c]	0.34	0.73	0.85[e]	0.58	0.4[b]	0.31[e]	0.08
	1m	–	4.58[c]	2.22	–	0.96	0.88[a]	–	0.92 [a,e]	0.73[a]
	6m	–	1.93	1.18	–	0.96	0.85[a]	–	0.77[a]	0.60[a]
14	0m	1.06	2.30[c]	0.73	0.77	0.85[e]	0.60	0.47	0.65	0.44
	1m	–	18.53 [c]	7.88	–	1.00	0.90[a]	–	1.00 [a,e]	0.79[a]
	6m	–	11.36	5.44	–	1.00	0.90[a]	–	1.00 [a,e]	0.81[a]
18C	0m	0.67	0.90[c]	0.35	0.73[b]	0.81[e]	0.50	0.37	0.50[e]	0.25
	1m	–	8.58[c]	2.51	–	0.96	0.85[a]	–	0.96 [a,e]	0.75[a]
	6m	–	3.93[c]	1.31	–	0.92	0.81[a]	–	0.85 [a,e]	0.60[a]
19A	0m	2.22	2.72	1.97	0.97	0.96	0.92	0.8	0.85	0.81
	1m	–	10.49[c]	7.14	–	1.00	0.98	–	1.00[a]	0.94[a]
	6m	–	5.80	4.46	–	1.00	0.98	–	0.92	0.92
19F	0m	1.10	1.43[c]	0.66	0.87	0.89	0.77	0.53	0.58	0.40
	1m	–	8.56	4.06	–	1.00	0.96[a]	–	0.96[a]	0.88[a]
	6m	–	4.19	2.49	–	1.00	0.94[a]	–	0.88[a]	0.75[a]
23F	0m	0.65[f]	0.62[c]	0.29	0.63[b]	0.69[e]	0.38	0.37[b]	0.35[e]	0.13
	1m	–	17.09[c,d]	2.12	–	1.00[a]	0.81[a]	–	1.00 [a,e]	0.69[a]
	6m	–	7.25[c,d]	1.17	–	1.00[a]	0.75[a]	–	0.92 [a,e]	0.56[a]

Chi-square and Fisher's exact tests were used to compare baseline proportions above a cut-off between groups. One-way analysis of variance (ANOVA) with Bonferroni correction was used to compare log-transformed antibody concentrations at baseline between groups. Chi-square was used to compare proportions above a cut-off between groups at each time- point after immunization. McNemar's test was used to detect changes in proportion from baseline within groups. Repeated-measures ANOVA and analysis of covariance were used to compare log-transformed antibody concentrations between and within group. Post-hoc analyses for simple effects were then performed as appropriate. 0m, baseline; 1m, 1 month after immunization; 6m, 6 months after immunization; AHC, adult healthy control; CHC, child healthy control; CP, child patient; ST, serotype.
[a]Proportion above cut-off significantly different before vs. after immunization ($P < 0.05$).
[b]Proportion above cut-off significantly different CHC vs. CP ($P < 0.05$).
[c]GMC significantly different AHC vs. CP ($P < 0.05$).
[d]GMC significantly different AHC vs. CP after adjusting for baseline ($P < 0.05$).
[e]Proportion above cut-off significantly different AHC vs. CP ($P < 0.05$).
[f]GMC significantly different CHC vs. CP ($P < 0.05$).
[g]GMC significantly different CHC vs. AHC ($P < 0.05$).

0.35 μg/ml was significantly lower in CP than in CHC for serotype 18C, whereas proportions above 1 μg/ml were significantly lower in CP than in CHC for two serotypes (3B and 6B). Comparing CHC with AHC, there was no significant difference in GMCs, proportions above 0.35 μg/ml and proportions above 1 μg/ml apart from for serotype 7F for which GMC was lower in CHC than in AHC.

Serology at 1 and 6 months

Geometric mean antibody concentrations and the proportions above each cut-off at 1 and 6 months after immunization are summarized in Table 2. GMCs for all serotypes were significantly higher than baseline at both time-points for both AHC and CP. GMCs were significantly higher for AHC group than for CP at 1 month for eight of 13 serotypes (6A, 6B, 7F, 9V, 14,

18C, 19A, 23F) and remained significantly higher at 6 months for five of 13 serotypes (6A, 6B, 7F, 18C, 23F). After adjusting for baseline antibody concentrations, GMCs were significantly higher in AHC than in CP for serotypes 6A, 6B and 23F at both 1 and 6-month time-points.

Comparing proportions above 0.35 μg/ml at baseline with 1-month results, there was a statistically significant increase for 11 of 13 serotypes for CP and five of 13 serotypes for AHC. For serotypes in which the increase in proportion above 0.35 μg/ml was non-significant, baseline proportions were already high and increased to 1 or just below. Increases in the proportions above 0.35 μg/ml persisted till 6 months for the majority of the serotypes. There was a significant increase in proportions above 1 μg/ml for all serotypes in both groups at the 1-month time-point. The proportions above 1 μg/ml were significantly higher for AHC than for CP at 1 month after immunization for six of 13 serotypes (6A, 6B, 9V, 14, 18C, 23F) and five of 13 at 6 months (6A, 6B, 14, 18C, 23F).

Factors predictive of 1-month antibody response in children with perinatally acquired HIV

For results of analysis of factors predictive of 1-month antibody response in children with paHIV, see Table, Supplemental Digital Content 1 (http://links.lww.com/QAD/A548). There was a highly significant association between higher baseline antibody levels and higher 1-month antibody levels for all 13 serotypes ($P < 0.05$). After adjusting for baseline antibody concentration, detectable viraemia was significantly associated with lower 1-month antibody for 11 of 13 serotypes ($P < 0.05$) and older age was significantly associated with lower 1-month antibody response for six of 13 serotypes ($P < 0.05$). After adjusting for age, baseline and viraemia, higher percentage of life with undetectable viral load was associated with higher 1-month antibody responses for three of 13 serotypes (4, 19F, 23F) ($P < 0.05$). No significant effect of prior PCV or PPV was found and there was no association between any LSS and magnitude of response.

Baseline opsonophagocytic assay comparison between children with perinatally acquired HIV and healthy children

In order to investigate naturally acquired immunity to serotypes found in PCV13 and not PCV7, we assessed OPA in CP and CHC groups at baseline. OPA was performed on serum samples from all children aged above 8 years for serotypes 1, 5 and 7F. For serotype 1 and 5, OPA titre was at least 8 in only four of 37 (11%) and one of 37 (3%) CP, respectively, and two of 17 (12%) CHC for both serotypes, despite a substantial proportion having antibody concentrations above 0.35 μg/ml. Conversely, for serotype 7F, 26 of 37 (70%) CP and 16 of 17 (94%) CHC had an OPA titre at least 8, respectively ($P = 0.08$). In view of these findings, further analysis of baseline OPA data was only possible for serotype 7F (summarized in Figure, Supplemental Digital Content 2, http://links.lww.com/QAD/A548). OPA geometric mean titer (GMT) was significantly higher in CHC [GMT = 1540, 1540, 95% confidence interval (CI) 621–3825)] than in CP (GMT = 246, 95% CI 96–631, $P = 0.02$). This was non-significant after adjusting for age, $CD4^+$ percentage and baseline serotype-specific IgG concentration. Regression analysis found no correlation between serotype-specific IgG concentration and OPA titre for healthy children. After adjusting for age, there was a significant positive correlation between log-transformed serotype-specific IgG concentration and OPA titre for the CP (regression coefficient = 1.17, 95% CI 0.44–1.90, $P = 0.003$). After adjusting for age and serotype-specific IgG concentration, viral load below 50 copies/ml was strongly associated with a higher OPA titre (antilog regression coefficient = 17.0, 95% CI 2.9–99.3, $P = 0.003$).

One-month opsonophagocytic assay comparison for children with perinatally acquired HIV and healthy adults

Opsonophagocytic assay was performed for serotypes 1, 5, 7F and 19A (serotypes in PCV13 but not PCV7) for both vaccinated groups, 1 month following vaccination. A significantly higher proportion of AHC had an OPA titre at least 8 for serotypes 1 and 5. For serotypes 7F and 19A, all AHC had an OPA titre at least 8, whereas, for CP, 45 of 47 had an OPA titre at least 8 ($P = 0.54$) (Fig. 3).

Comparing OPA GMT, 1 month following vaccination between AHC and CP showed borderline significantly higher OPA GMT for AHC for serotypes 1 ($P < 0.05$), 5 ($P = 0.06$) and 7F ($P < 0.05$). After adjusting for serotype-specific IgG and $CD4^+$ percentage, these differences were non-significant. For both groups, regression analysis revealed a significant positive correlation between serotype-specific log-transformed IgG concentration and OPA titre for all the four serotypes for CP and three of four serotypes for AHC. Univariate analysis of the CP results showed viral load below 50 copies/ml to be strongly associated with higher OPA titre for all the four serotypes assessed ($P < 0.005$). This effect remained significant after adjusting for age and serotype-specific IgG concentration for three of four serotypes (5, 7F, 19F) ($P < 0.05$).

Discussion

To our knowledge, this is the first study to present detailed immunogenicity data for PCV13 in an HIV-infected patient cohort. We measured serum IgG to all 13 vaccine serotypes before and after immunization with a single dose of PCV13. Serotype-specific IgG was present at

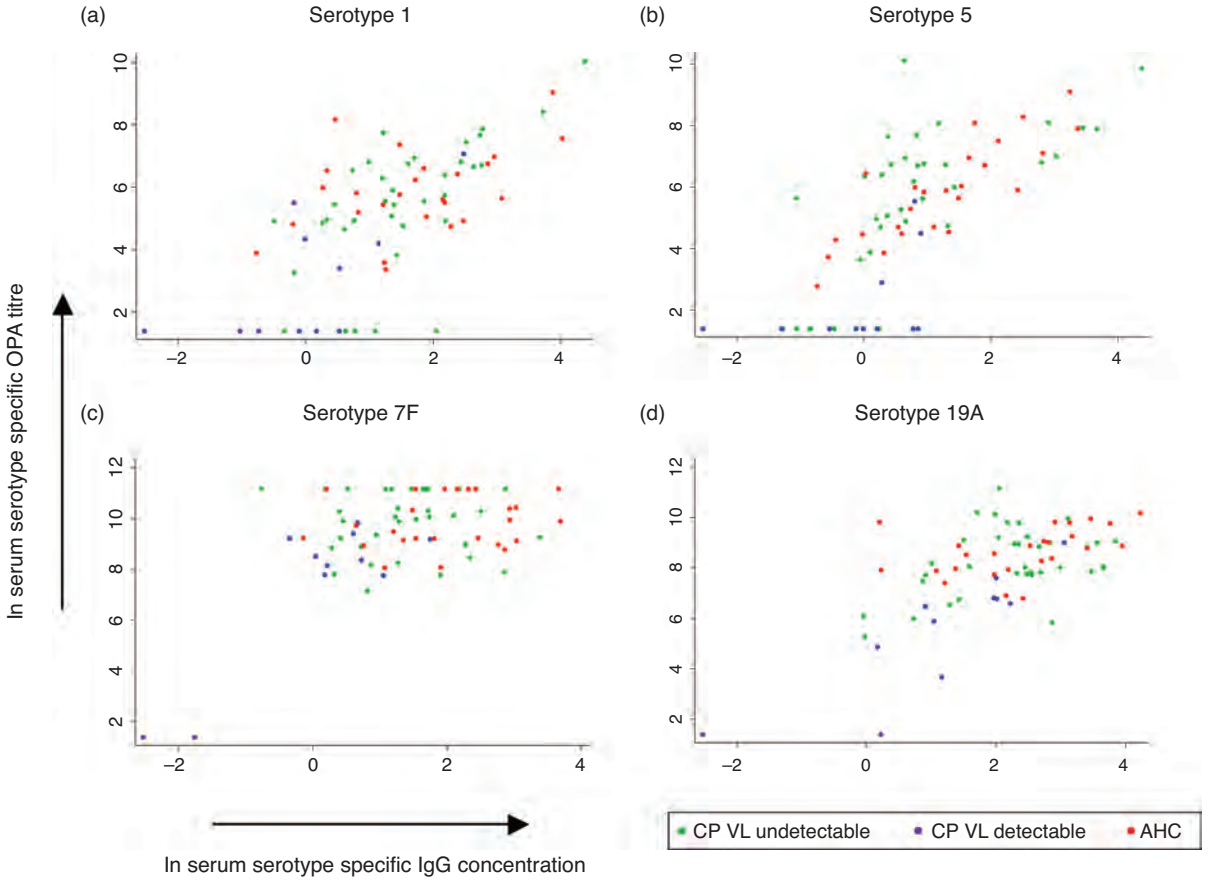

Fig. 3. Scatter graphs showing log-transformed opsonophagocytic assay titres against log-transformed serotype-specific IgG for adult healthy controls and child patients 1 month after 13-valent pneumococcal conjugate vaccine. Grey vertical lines indicate transformed value for the cut-offs of 0.35 and 1 μg/ml. 1.39 on the Y-axis corresponds to an OPA titre of 4. IgG concentration significantly correlated with OPA titre for all serotypes in both groups except 7F for AHC. Linear regression was used to test for association between log-transformed OPA and IgG concentration. Chi-square and Fisher's exact tests were used to compare proportions. Serotype 1: CP ($P < 0.005$, R2 = 0.51); AHC ($P < 0.05$, R2 = 0.16). Serotype 5: CP ($P < 0.005$, R2 = 0.48); AHC ($P < 0.005$, R2 = 0.70). Serotype 7F: CP ($P < 0.005$, R2 = 0.29); AHC ($P = 0.4$, R2 = 0.01). Serotype 19A: CP ($P < 0.005$, R2 = 0.35); AHC ($P < 0.05$, R2 = 0.18). A higher proportion of children with paHIV had OPA titres below 8 for serotypes 1 [AHC: 25/25 (100%) vs. CP: 36/47 (77%), $P = 0.007$] and 5 [AHC: 25/25 (100%) vs. CP: 32/47 (68%), $P = 0.001$], despite the majority having serotype-specific IgG concentrations greater than 0.35 μg/ml. AHC, adult healthy control; CP, child patient; VL undetectable, viral load below 50 copies/ml; VL detectable, viral load above 50 copies/ml.

baseline, although this did not consistently correspond to detectable OPA activity. Children with paHIV mounted relatively robust 1-month serotype-specific IgG responses, which persisted to 6 months for the majority of serotypes. Higher baseline IgG concentration was associated with higher 1-month antibody levels, and serum IgG correlated well with OPA following vaccination. Ongoing HIV viraemia was associated with an impairment of both IgG and OPA responses.

The presence of serotype-specific IgG in unvaccinated individuals is considered to be the result of natural exposure through pneumococcal carriage [21,22]. We found baseline IgG concentrations for healthy and HIV-infected children to be broadly similar for the majority of serotypes, consistent with existing reports [5–7,9,11], suggesting increased susceptibility to IPD in paHIV might not be attributable to lower levels of naturally acquired circulating IgG. However, absence of detectable functional antibody (as measured by OPA activity) in both healthy and HIV-infected children despite measurable naturally acquired IgG by ELISA suggests that IgG measurement by ELISA in unvaccinated individuals may not adequately predict protection. Nevertheless, baseline IgG concentrations strongly predicted 1-month antibody concentrations following vaccination. The mechanism by which naturally acquired serological immunity develops with age is not clear, especially for those invasive serotypes that rarely colonize. It is possible that steady-state IgG concentrations are a reflection of cumulative exposure to pneumococcal antigens and other immune stimuli resulting in persistent polysaccharide-specific IgG

production by plasma cells. This might then be a proxy measure for the integrity of the immune system at times of past antigen exposure and also of the potential for 'boostable' memory response following immunization.

Geometric mean concentrations following immunization were lower in HIV-infected children than healthy young adults for a number of serotypes. After adjusting for baseline antibody concentration, the response to three serotypes (6A, 6B and 23F) remained relatively impaired. These serotypes are particularly associated with IPD in HIV-infected adults and children [23–25]. These observations support the hypothesis that HIV infection impacts upon acquired immunity in such a way as to affect the immune response to some pneumococcal serotypes more than others. In healthy individuals, serotype-specific IgG responses to PCV vary according to serotype, despite uniform T-cell responses to vaccine carrier protein [26]. It has been suggested that this may be a result of variations in B-cell repertoire [26]. Reports from our own group and others have demonstrated alterations in B-cell phenotype in the context of HIV [27–36], which can be related to both naturally acquired and vaccine-induced pneumococcal immunity [27,32,34]. It would be informative to investigate how detailed B and T-cell phenotype might relate to our read-outs of vaccine immunogenicity.

A cut-off of 0.35 μg/ml is used as a correlate of protection against IPD in infant PCV immunization [20]. Its utility as a correlate for protection in other age and risk groups has not been established. Our observation that many adults and children have IgG concentrations above this cut-off prior to immunization suggests that its usefulness in this context is limited. Correlates of vaccine-induced pneumococcal immunity outside of infancy and in risk groups are needed and require large-scale efficacy studies, which are much less feasible following the success of PCV in reducing the burden of IPD. The results of the Community Acquired Pneumonia IMmunization Trial in Adults (CAPITA), a large-scale randomized trial investigating efficacy of PCV13 in adults above 65 years of age (http://clinicaltrials.gov/show/NCT00744263) may provide some data to this effect, but will still not be directly applicable to adolescent risk groups. The use of protection against pneumococcal carriage as a surrogate for protection against IPD is currently being explored and will hopefully provide alternative measurable correlates of protection [37].

Dosing schedule and timing of vaccination with PCV for children with paHIV is currently debated [13,16–19]. Some argue that in the context of universal infant immunization, PCV is not indicated due to efficient herd immunity [17]. However, herd immunity is not as effective in HIV-infected populations [38]. Our data on the immunogenicity of a single dose of PCV13 in this older age group can inform guidelines for its use in this context and also, perhaps more importantly, in settings where herd immunity is a long way from being established and where the numbers of older children and adolescents with HIV are high and increasing. Lower-valency PCVs have been introduced into national schedules worldwide. Immunogenicity data in a range of ages and risk groups and systematic monitoring for serotype replacement will both be important when considering the need for future introduction of higher-valency vaccines.

We found immunogenicity to be limited in the context of viraemia, consistent with the immunogenicity studies of other vaccines in paHIV (reviewed in [39]) and supporting recommendations to vaccinate once fully suppressed on HAART and to consider revaccination/boosting if primary vaccination occurred while being viraemic [18]. When to start HAART in children beyond infancy is also a subject to debate globally [40]. Recently updated WHO guidelines recommend HAART for all HIV-infected children under 5 years of age [41]. Although there are likely differences between our UK cohort and HIV-infected children in resource-limited settings (e.g. higher BMI, less OI), our data support the argument that HAART should be initiated at the earliest opportunity in order to optimize immune responses to both natural infection and vaccine antigens. This is especially relevant in resource-limited settings given the relatively high exposure to infectious pathogens.

Our study has some limitations and we thus plan to study in the future further time-points at 1 year or beyond, the use of a booster dose to examine immune memory and antigen-specific in-vitro B-cell studies. We were limited ethically to recruiting healthy young adult controls rather than age-matched controls, which may have helped elucidate further differences between healthy and HIV-infected children.

In conclusion, we have provided the first detailed immunogenicity data for PCV13 in HIV-infected children. This will both inform vaccination practices and HAART treatment strategies in this unique and vulnerable group.

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A.B. conceived and designed the study with advice and supervision from B.K., D.G. and P.K. A.B. applied for and obtained funding and ethical approval for the study. H.L. facilitated collaboration between academic and clinical services and advised on study design. A.B., M.H. and M.Z. performed all laboratory work apart from that performed in routine NHS laboratory. A.B. recruited all study participants and collected and analysed all data. A.B. prepared the manuscript with supervision from B.K. and

input from D.G., P.K., H.L., M.H. and M.Z. All authors approved the final manuscript.

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Conflicts of interest

B.K. has performed consultancy work on behalf of Pfizer and has ongoing investigator-initiated proposals and ongoing trials involving PCV13. D.G.'s laboratory (including M.H. and M.Z.) performs contract and or collaborative research for/with GlaxoSmithKline, Sanofi Pasteur and Merck. D.G. has received travel or honorarium support for participation in external expert committees for Merck, Pfizer, Sanofi Pasteur and GlaxoSmithKline. P.K. has received grants from Pfizer and Roche. A.B. and H.L. declare no conflict of interest.

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