Published in final edited form as:

J Infect Dis. 2010 August 15; 202(3): 355–361. doi:10.1086/653704.

Effect of HIV infection status and anti-retroviral treatment on quantitative and qualitative antibody responses to pneumococcal conjugate vaccine in infants

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Abstract

Serotype specific antibody concentration and opsonophagocytic activity (OPA) were evaluated following three doses of pneumococcal conjugate vaccine. Groups included HIV+ infants with CD4+ $\geq 25\%$ initiated on immediate antiretroviral treatment (HIV+/ART+) or ART was deferred until clinically or immunologically indicated (HIV+/ART-). Immune responses were also evaluated in HIV non-infected infants born to HIV-seronegative (M-/I-) or HIV+ mothers (M+/I-). Antibody concentrations were similar between HIV+/ART+ and HIV+/ART- infants. Antibody concentrations were, however, lower in M-/I- compared to M+/I- infants. M-/I- infants, nevertheless, had superior OPA responses compared to HIV+/ART+, who in turn had better OPA responses compared to HIV+/ART- infants.

Keywords

Streptococcus pneumoniae; pneumococcal conjugate vaccine; HIV exposure; opsonophagocytic assay

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Conflict of interest: Receipt of research funding and consultancy from Wyeth Vaccines and Pediatrics (SAM, KPK). Receipt of research grants, consultancies and honoraria from GSK (HK, SAM, KPK). Prevnar® was donated by Wyeth.

Meeting presentations:

The results of this study were presented at the 5th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention in Cape Town, South Africa from 19–22 July 2009. Abstract numbers: MOPEB023 and MOPEB024.

Background

Conflicting evidence of the immunogenicity of pneumococcal conjugate vaccine (Vaccine) in HIV infected infants may be due differences in dosing schedules, Vaccine formulation, vaccination age, degree of immunosuppression and antiretroviral treatment (ART) access.^{1–3} Additionally, there is only limited data on functional activity of antibody induced by Vaccine in HIV infected infants with one study demonstrating functionally impaired antibody in HIV infected children not receiving ART.³

Although maternal transmission of HIV to infants is steadily declining, HIV non-infected infants are also at increased risk of morbidity and mortality,⁴ suggesting possible immune suppression.

This study examined the effect of: i. early versus deferred ART initiation in HIV infected infants categorized as immunocompetent and; ii. in-utero and perinatal exposure to HIV in HIV non-infected infants, on antibody concentrations and opsonophagocytic activity (OPA) following pneumococcal conjugate vaccination in early infancy.

Methods

The study enrolled *a priori* four groups of children aged 6 to 12 weeks. These included 2 groups of HIV infected infants, co-enrolled from the Children with HIV Early Antiretroviral (CHER) Study in South Africa, 5 with CD4+ T-lymphocyte cells $\geq 25\%$ randomized to initiate ART immediately (HIV+/ART+ group); or ART was initiated when clinically or immunologically indicated (HIV+/ART– Group).⁶ The ART regimen included zidovudine, lamivudine and lopinavir/ritonavir. Additionally, two cohorts of HIV non-infected infants were prospectively enrolled in parallel to the HIV infected children including: i. infants born to HIV infected mothers who were HIV PCR (Roche Amplicor Version 1.5 RNA PCR) negative at baseline and one month after the third dose of Vaccine (M+/I–) and ii. infants born to mothers seronegative for HIV after 24 weeks of gestational age during pregnancy and who were HIV ELISA seronegative at study-enrolment (i.e. M–/I–).

Additional participant-eligibility criteria included absence of intercurrent illness within 72 hours of enrolment, no Grade 3 or 4 clinical or laboratory toxicity as per DAIDS Pediatric Adverse Experiences,⁷ birth weight of at least 2000 grams, participation in the CHER study for HIV infected infants, absence of receipt of any blood products prior to study entry, any immunomodulating medication for more than two weeks within one week of possible enrolment

Infants were enrolled between April 2005 and June 2006 and scheduled to receive three doses of 7-valent pneumococcal conjugate vaccine (i.e. Prevnar®; Wyeth Vaccines, NJ, USA) at 6 to 12, 9 to 18 and 12 to 24 weeks of age. Infants received other scheduled childhood vaccines, included in the public immunization program, concurrently with Prevnar®.

Immune response to the primary series of Vaccine was measured 3 to 6 weeks after the third dose using serum from venous blood which had been centrifuged, aliquotted and stored at –20 to –70°C until processing at the Respiratory and Meningeal Pathogens Research Unit (RMPRU), Johannesburg, South Africa. A standardized enzyme immunoassay (EIA), including adsorption with 22F polysaccharide, was used to test for vaccine-serotype specific capsular IgG antibody concentrations as described.^{8 9}

The functionality of the antibodies post vaccination was determined by opsonophagocytic killing assay (OPA) for serotypes 9V, 19F and 23F using differentiated HL-60 cells as described.^{8 10} Lower antibody concentrations required for 50% killing activity on OPA is suggestive of superior antibody functional activity. Detectable killing activity on OPA was defined as a titer of ≥ 8 .

For quality assurance, a quality control serum from a vaccinated volunteer was included on each plate. The coefficient of variation for the control sera were $<40\%$ for all serotypes.

Statistical analysis

Data were analyzed using SAS® 9.1 (SAS Institute Inc., Cary, NC, USA.). The geometric mean concentrations and 95% confidence intervals (95% C.I.) of serotype-specific antibody concentrations and opsonophagocytic titers were calculated following $\log_{10}$ transformation of the data. Comparisons of mean concentration were performed using analysis of covariance (ANCOVA) on $\log_{10}$ transformed data with study center, gender, race, and baseline value of the antibody concentration or opsonophagocytic titer as covariates.

Logistic regression with study center, gender, and race as covariates was applied for comparisons of the proportion of children between groups with serum antibody thresholds of $\geq 0.35 \mu\text{g/ml}$. This has been suggested as a putative measure of community immunity against invasive pneumococcal disease.¹¹

The functionality of antibodies in serum samples was expressed as opsonophagocytic titer, which is the reciprocal of the serum dilution determined to induce 50% killing of pneumococci. If the observed killing of pneumococci by HL-60 cells at the lowest dilution (1:8) was less than 50%, a value of 4 was assigned. The antibody concentration required for 50% killing was calculated by dividing the IgG serotype specific antibody concentration by the OPA titer. The correlation between the serotype specific IgG antibody concentration and OPA titers was measured using Pearson's correlation coefficients. An α value of ≤ 0.05 was considered significant.

To minimize confounders between groups, the data were analyzed based upon infants receiving the primary series of Vaccine and the immunogenicity bloods being collected within the protocol specified window periods, i.e. per protocol analysis.

Ethics Considerations

This study was approved by the Human Subjects Research Committees of the University of the Witwatersrand, Stellenbosch University, Medicine Control Council of South Africa and Clinical Science Review Committee of the Division of AIDS. Signed informed consent was obtained from the parents of the children for participation in this study. The clinical trials registry reference number for this study is ClinicalTrials.gov NCT00099658 and for the CHER study NCT00102960.

Results

A total of 565 infants were enrolled with 81.9% of 210 HIV+/ART+, 73.3% of 105 HIV+/ART-, 96.0% of 125 M+/I- and 91.2% of 125 M+/I- included in the analysis. The major factor for non-inclusion in the immunogenicity analysis among HIV infected children was the higher mortality rate in HIV+/ART- (10.5%) compared to HIV+/ART+ (2.4%; $P=0.004$) as reported.⁵

The mean age of receipt of the three Vaccine doses and timing of the immunogenicity visit did not differ between groups (Table 1). Additionally, all HIV+/ART+ infants had been initiated on ART within 4 days preceding the first dose of Vaccine and 13 (16.8%) HIV+/ART- infants had ART initiated prior to immunogenicity visit at a mean of 40 days following the first dose of Vaccine (Table 1). IgG antibody concentrations and OPA titers did not differ by ART status when measuring immunogenicity in the HIV+/ART- infants (data not shown).

Post-primary series quantitative antibody responses to PCV-7

Greater than 92% of subjects in all groups had IgG antibody concentration ≥ 0.35 $\mu\text{g/ml}$ for all serotypes, except for serotype 6B in HIV+/ART+ (88.3%) and M-/I- (82.5%) infants (Table 2).

Antibody concentrations and proportions of infants with antibody concentration ≥ 0.35 $\mu\text{g/ml}$ were similar between HIV+/ART+ compared to HIV+/ART- infants, except antibody concentrations for serotypes 6B and 14 which were lower in HIV+/ART+ infants (Table 2).

Antibody concentrations and the proportion of infants with concentrations ≥ 0.35 $\mu\text{g/ml}$ were significantly higher for serotype 6B in HIV+/ART+ infants compared to M-/I- infants. A similar trend of higher antibody concentrations in HIV+/ART+ compared to M-/I- infants were observed for other serotypes, except serotype 4. Although antibody concentration were also greater for serotypes 6B and 14 in HIV+/ART- compared to M-/I- infants, there was no consistent pattern in the direction of differences in antibody concentrations for other serotypes (table 2).

A similar proportion of M+/I- compared to M-/I- infants had antibody concentrations ≥ 0.35 $\mu\text{g/ml}$ for each serotype, except serotype 6B which was greater in M+/I- infants. The antibody concentrations were, however, generally higher in M+/I- infants compared to M-/I- infants, including significant differences for serotypes 6B and 23F (Table 2).

Opsonophagocytic activity assay responses

A lower proportion of HIV+/ART- infants had OPA titers ≥ 8 for serotypes 9V and 19F (and a similar trend for 23F) compared to HIV+/ART+ infants (Table 2). Additionally, OPA titers were significantly lower and higher antibody concentration was required for 50% killing activity for all serotypes in HIV+/ART- compared to HIV+/ART+. Similarly, inferior OPA responses were observed on HIV+/ART- compared to M-/I- infants (Table 2).

There were no differences in OPA titres between HIV+/ART+ compared to M-/I- infants, apart from HIV+/ART+ infants requiring higher antibody concentration for 50% killing on OPA for serotypes 19F and 23F (Table 2). Additionally, except that M+/I- infants required higher concentration of antibody for 50% killing activity against serotype 19F, there were no other differences observed in OPA measurement between M+/I- and M-/I- infants (Table 2).

There was significant correlation between serotype-specific antibody concentration and OPA for all three studied serotypes in all four groups ($P < 0.0001$ for all comparisons; Table 2).

Discussion

To our knowledge, this is the first systematic evaluation of the effect of timing of ART in HIV infected children on immune responses to a primary series of pneumococcal conjugate

vaccine. Similarly, this is the first study to report on the effect of perinatal HIV exposure on immune responses to Vaccine in HIV non-infected infants.

Although the timing of ART initiation did not affect quantitative antibody responses between HIV+/ART+ and HIV+/ART- infants, delaying ART initiation was associated with functionally impaired antibody responses measured by OPA. There is no established OPA assay associated correlate of protection against pneumococcal disease, however, the poorer OPA activity indicates immunological abnormalities in HIV+/ART- compared to HIV+/ART+ infants. This occurred despite a baseline CD4+ percentage of 37.7%, considered to signify immunocompetence in HIV+/ART- infants. These findings provide insight into immune aberrations that may exist in HIV+/ART- infants and provide a further biological reason for the heightened morbidity and mortality observed in CHER when ART was deferred. 5

Whilst higher antibody concentrations were observed in HIV+/ART+ compared to M-/I- infants, the qualitative immune response to Vaccine indicated that higher concentration of antibody for 50% killing on OPA was required for two serotypes in HIV+/ART+ infants. Whereas the clinical relevance of this difference is uncertain, it may suggest that even early ART initiation does not fully restore the immune responses to Vaccine in HIV+/ART+ infants.

The importance of measuring OPA in addition to antibody concentrations is increasingly supported by the recognition that serotype specific antibody protects against pneumococcal disease through opsonizing pneumococci and facilitating phagocytosis. The results both from our previous study and this study are supportive, 3 indicating that relying solely on antibody concentration above a certain threshold may inadvertently over-estimate the likely protection of Vaccine in HIV infected infants. The mechanism for diminished OPA response in the HIV infected infant groups may relate to compromised maturation and antibody avidity and is currently under study.

While *in-utero* HIV exposure in HIV non-infected children has also been associated with excess morbidity and mortality, the biological reason is yet to be fully characterized. Aberrations of the immune system of M+/I- children have been described, 12 albeit, conflicting data regarding immune responses to BCG vaccine in these children^{13 14} The only inferior immune response observed in our study in M+/I- compared to M-/I- infants related to higher antibody concentration required for 50% killing on OPA against serotype 19F. This may suggest that subtle changes in the immune response to vaccines in M+/I- infants could contribute to the increased morbidity and mortality in these children.

Surprisingly, IgG antibody concentration following vaccination were generally higher in M+/I- compared to M-/I- infants, indicating more robust rather than dampened quantitative antibody response in M+/I- infants. Possible contributory factors currently under study include the likelihood that higher transplacental transmission of serotype-specific antibody in M-/I- compared to M+/I- infants may be associated with dampening the immune responses to Vaccine in M-/I- infants. Additionally, possible differences in Vaccine related serotype nasopharyngeal colonization between M-/I- and M+/I- infants at the time of immunization, which has been negatively associated with quantitative antibody responses,¹⁵ are currently being evaluated.

Limitations of our study include that the magnitude of the poorer immune responses in HIV +/ART- may have been under-estimated, since immunogenicity of the Vaccine may have been weaker in infants who died prior to the immune response being measured. Additionally, the objectives of the study were specific to HIV infected children with CD4+ $\geq 25\%$ and the results may not be extrapolatable to HIV infected children with lower CD4+

counts. Furthermore, unmeasured factors between M⁻/I⁻ and M⁺/I⁻ may have contributed to differences in the quantitative antibody responses between these children.

Acknowledgments

Support for this study was provided by the National Institute of Allergy and Infectious Diseases (NIAID) of the US National Institutes for Health (NIH), through the Comprehensive International Program of Research on AIDS (CIPRA) network, grant number U19 AI53217. Additional support for this work was provided with Federal funds from the National Institute of Allergies & Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN272200800014C. Collaborators and Centers for study: South Africa: Avy Violari, Ronelle van Niekerk, James McIntyre, Wilma Pelsler, Aneesa Naem Sheik, Melissa Budge, Munira Saleh, Sindile Mashinini, Sibongile Dlamini, Valerie Kemese, Jean Bolton (Perinatal HIV Research Unit); Mark F Cotton, Helena Rabie, Anita Janse van Rensburg, Els Dobbels, George Fourie, Marietjie Bester, Wilma Orange, Ronelle Arendze, Catherine Andrea, Marelize Smuts, Kurt Smith, Theresa Louw, Alec Abrahams, Kenny Kelly, Amelia Bohle, Irene Mong, Jodie Howard, Tanya Cyster, Genevieve Solomon, Galroy Benjamin, Jennifer Mkalipi, Edward Barnes (Children's Infectious Diseases Clinical Research Unit); Glenda Gray, Ian Sanne, Ravindre Panchia, Christie Davies, Morna Cornell (CIPRA-SA); Peter Adrian; Shabir A Madhi; Nadia van Niekerk (Respiratory and Meningeal Pathogens Research Unit)

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Table 1

Demographic and baseline features of participants included in the analysis evaluating the immunogenicity of a 7-valent pneumococcal conjugate vaccine (PCV-7).

Variable	HIV+/ART+ ^a	HIV+/ART- ^b	M+/I- ^c	M-/I- ^d
Number of subjects ^e	172	77	120	114
Male sex	68 (39.5)	29 (37.7)	65 (54.2)	61 (53.5)
Race: Black Africa Mixed ancestry	164 (95.3) 8 (4.7)	76 (98.7) 1 (1.3)	112 (93.3) 8 (6.7)	86 (75.4) 28 (24.6)
Mean age (wks) at dose 1 (Standard deviation; SD)	7.5 (1.2)	7.3 (1.2)	7.4 (1.0)	7.0 (1.0)
Mean age (wks) at dose 2 (SD)	11.5 (1.3)	11.4 (1.3)	11.5 (1.0)	11.1 (1.1)
Mean age (wks) at dose 3 (SD)	15.5 (1.3)	15.5 (1.3)	15.5 (1.0)	15.2 (1.2)
Mean (days) of immunogenicity visit and dose 3 (SD) ^f	28.3 (2.1)	29.2 (4.7)	28.0 (2.3)	28.1 (2.0)
Median CD4+ lymphocyte Count at Baseline (Interquartile range; IQR)	2 083.5 (1569.0 to 2747.0)	2 044.0 (1584.0 to 2796.0)	Not indicated	
Mean CD4+ lymphocyte % at Baseline (SD)	34.9 (8.1)	37.7 (8.9)		
Median CD4+ lymphocyte count at immunogenicity visit (IQR)	2210.0 (1703.0 to 2933.0)	1 862.0 (1112.0 to 2584.0)		
Mean CD4+ % at immunogenicity visit (SD)	40.8 (9.4)	30.5 (9.2)		
Median HIV Viral Load (copies/ml) at Baseline (IQR)	>750 000 (377000 to >750000)	>750 000 (411 000 to >750000)		
Median days (range) to ART initiation relative to Dose 1	0 (-4 to 0)	40 (7.0 to 71.0) ⁷		

^a HIV+/ART+ = HIV infected children with CD4+ cell count ≥25% started on antiretroviral treatment (ART) at time of first dose of PCV-7.

^b HIV+/ART- : HIV infected children with CD4+ cell count ≥25% at time of first PCV-7 dose that were started on ART based on clinical or immunological indications.

^c M+/I-: HIV non-infected children born to HIV infected mothers.

^d M-/I- = HIV non-infected children born to HIV non-infected mothers.

^e Includes subjects who received all PCV doses within protocol-defined window periods and available immunogenicity data within 3–6 weeks after 3rd dose of PCV-7.

^f Immunogenicity visit refers to visit undertaken 3–6 weeks following 3rd dose of PCV-7.

⁷ 13 (16.8%) of 77 HIV+/ART- children had been initiated on ART at the time of the immunogenicity analysis.

Table 2

Quantitative and qualitative serotype-specific antibody responses one month following a primary series of 3 doses of pneumococcal conjugate vaccine (PCV).

Serotype	Measure	HIV+/ART+ ^a N=172	HIV+/ART- ^b N=77	P value ^g	M+/I- ^c N=120	M-/I- ^d N=114	P value h	P value i	P value ^j
4	GMC 95% CI ^e	5.74 (5.00; 6.57)	5.94 (4.41; 8.00)	0.83	7.99 (7.00; 9.12)	6.45 (5.32; 7.82)	0.14	0.21	0.40
	% ≥0.35µg/ml ^f	99.4	97.4	n/c ^k	100	98.2	n/c	n/c	n/c
6B	GMC	2.25 (1.84; 2.74)	3.52 (2.43; 5.10)	0.027	2.60 (2.01; 3.37)	1.43 (1.05; 1.94)	0.025	0.051	<0.001
	% ≥0.35µg/ml	92.4	88.3	0.30	92.5	82.5	0.032	0.02	0.33
9V	GMC	5.48 (4.82; 6.23)	4.85 (3.62; 6.50)	0.29	6.45 (5.64; 7.38)	5.12 (4.36; 6.0)	0.13	0.98	0.36
	% ≥0.35µg/ml	98.8	94.8	0.07	99.2	99.1	0.97	0.65	0.08
14	GMC	4.81 (4.08; 5.67)	6.33 (4.84; 8.28)	0.04	4.47 (3.67; 5.46)	3.60 (2.90; 4.47)	0.78	0.08	<0.001
	% ≥0.35µg/ml	99.4	97.4	0.18	98.3	96.5	0.58	0.20	0.86
18C	GMC	6.06 (5.30; 6.93)	5.20 (3.85; 7.03)	0.23	7.46 (6.50; 8.57)	6.02 (5.00; 7.25)	0.33	0.57	0.12
	% ≥0.35µg/ml	99.4	93.5	n/c	100	99.1	n/c	n/c	n/c
19F	GMC	7.55 (6.69; 8.53)	6.19 (4.87; 7.88)	0.13	8.47 (7.07; 10.14)	6.97 (5.88; 8.37)	0.15	0.56	0.39
	% ≥0.35µg/ml	100	97.4	n/c	99.2	99.1	n/c	n/c	n/c
23F	GMC	4.83 (4.03; 5.80)	5.38 (3.99; 7.44)	0.53	6.91 (5.84; 8.17)	4.10 (3.23; 5.19)	0.003	0.42	0.22
	% ≥0.35µg/ml	96.5	94.8	0.52	99.2	94.7	0.11	0.65	0.85
Opsonophagocytic activity analysis									
Serotype 9V OPA	Number (%) OPA ≥8 ^l	171 (99.4)	68 (88.3)	0.003	118 (99.2)	114 (100)	0.99	0.99	0.001
	OPA GMT (95% CI) ^m	725 (623; 843)	331 (219; 500)	<0.0001	610 (497; 749)	566 (471; 681)	0.67	0.09	0.0023
	Antibody (95% CI) for 50% killing ⁿ	7 (7; 9)	15 (11; 20)	<0.0001	11 (9; 12)	9 (8; 11)	0.23	0.13	0.0011

Serotype	Measure	HIV+/ART+ ^a N=172	HIV+/ART- ^b N=77	P value ^g	M+/I- ^c N=120	M-/I- ^d N=114	P value h	P value i	P value ^j
	ELISA:OPA correlation ^o	0.509	0.674	NA ^p	0.629	0.471	NA	NA	NA
Serotype 19F OPA	Number (%) OPA ≥8	169 (98.3%)	68 (88.3%)	0.003	115 (95.8%)	113 (99.1%)	0.15	0.54	0.0106
	OPA GMT (95%CI)	312 (267; 365)	149 (101; 218)	<0.0001	283 (230; 349)	365 (302; 439)	0.10	0.264	<0.0001
	Antibody (95%CI) for 50% killing.	24 (21; 27)	42 (31; 55)	<0.0001	30 (26; 35)	19 (16; 23)	0.0004	0.0454	<0.0001
Serotype 23F OPA	ELISA:OPA correlation	0.670	0.689	NA	0.691	0.425	NA	NA	NA
	Number (%) OPA ≥8	163 (95.3)	69 (89.6)	0.10	118 (98.3)	110 (97.3)	0.61	0.38	0.0355
	OPA GMT (95%CI)	1309 (1016; 1686)	758 (468; 1227)	0.015	2678(2133;3363)	1755 (1311; 2350)	0.052	0.13	0.0005
	Antibody (95%CI) for 50% killing.	4 (3; 5)	7 (5; 10)	0.0005	3 (2; 3)	2 (2; 3)	0.62	0.0068	<0.0001
	ELISA:OPA correlation	0.589	0.682	NA	0.588	0.443	NA	NA	NA

^a HIV+/ART+: HIV infected children with CD4+ cell count ≥25% started on antiretroviral treatment (ART) at time of first dose of PCV.

^b HIV+/ART-: HIV infected children with CD4+ cell count ≥25% at time of first PCV dose that were started on ART based on clinical or immunological indications.

^c M+/I-: HIV non-infected children born to HIV infected mothers.

^d M-/I-: HIV non-infected children born to HIV non-infected mothers.

^e GMC (95%CI) = Geometric mean antibody concentration and 95% confidence intervals in parenthesis.

^f Proportion of children with an antibody concentration ≥0.35 µg/ml.

^g Comparison of HIV+/ART+ to HIV+/ART-.

^h Comparison of M+/I- to M-/I-.

ⁱ Comparison of HIV+/ART+ to M-/I-.

^j Comparison of HIV+/ART- to M-/I-.

- ^k n/c= The p-value is not calculable through logistic regression as at least one of the groups have a value of 100%.
- ^l Infants with titers ≥ 8 on OPA assay. Value in parenthesis is a percentage of those tested which may have been less than the total in the group.
- ^m GMT (95%CI): Geometric mean titre of OPA and 95% confidence intervals in parenthesis.
- ⁿ Mean antibody concentration in ng/ml required for 50% killing activity on OPA calculated by the GMC of a newly created variable derived from dividing the serotype specific antibody concentration of a sample by the serotype-specific continuous opsonophagocytic tier of the sample.
- ^o Pearson's correlation coefficient of log transformed data for serotype specific ELISA and OPA. P value was <0.0001 for all correlation evaluations.
- ^p NA= not applicable