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Evaluation of humoral response to heptavalent pneumococcal conjugate vaccine in HIV-infected children

ABSTRACT

OBJECTIVE: Invasive pneumococcal disease is a major cause of death in HIV-infected children. The objective of the study was to assess the quantitative antibody response to the seven pneumococcal serotypes of heptavalent pneumococcal conjugate vaccine in a group of HIV-infected children.

METHODS: Study comprising 40 HIV-infected children aged between 2 and 9 years followed up in a specialized outpatient clinic in São Paulo, Brazil, between 2002 and 2003. Enzyme immunoassay (ELISA) was used to measure IgG antibody titers against pneumococcus capsule. Antibodies were measured immediately before and 1 month after the second dose of the vaccine. Two response criteria were used: IgG titers ≥1.3 μg/mL in the post-immunization serology and an increase of at least 4-fold in post- compared to pre-immunization serology.

RESULTS: For the first criterion (≥1.3 μg/mL), 26 (65%) children had serological response to the vaccine, 12 (30%) showed post-immunization IgG titers of at least 1.3 μg/mL for all seven serotypes studied. For the second criterion studied (≥4-fold increase in post- compared to pre-immunization titers for four serotypes or more), serological response was seen in 15 (37.5%) children.

CONCLUSIONS: Overall response to the heptavalent pneumococcal conjugate vaccine was adequate, showing a statistically significant increase in the post-immunization geometric mean titers for the seven serotypes studied.

DESCRIPTORS: Pneumococcal Vaccines. Immunologic Factors. Pneumococcal Infections, prevention & control. HIV Infections, prevention & control. Child.

INTRODUCTION

Invasive pneumococcal disease is a major cause of disease burden and death in HIV-infected children. The first studies to estimate the incidence rate and cause of bacterial disease in human immunodeficiency virus (HIV) infected children were conducted by late 1980s. In 1985, Bernstein et al[1] investigated 46 HIV-infected children and reported 27 episodes of sepsis in 21 of them and the most commonly seen pathogenic agent was *Streptococcus pneumoniae*. Krasinski et al[8] have also isolated *S. pneumoniae* in 31% of blood cultures of bacterial infections in HIV-infected children.

Farley et al,[3] in 1994, prospectively followed up a cohort of HIV-infected infants from birth and found an incidence of invasive disease of 11.3 per 100 chil-

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dren-year during the first three years of life compared to 0.5 in the control group. This incidence rate was considered high and the highest predisposition of HIV-infected population to invasive disease was attributed to the combination of two factors: delayed response to pneumococcal polysaccharide antigens (normally occurring in children under 18 to 24 months of age) and poor humoral immune response characteristically seen in HIV infection.

In a South African study carried out in 2000, Madhi et al[11] investigated children under 12 who had their blood or cerebrospinal fluid cultured. These authors found 237 cases of *S. pneumoniae* invasive disease, of which 64.9% were in HIV-infected children. They reported in this population a 41.7-fold increased risk (95%CI 26.5;65.6) of developing invasive pneumococcal infection compared to same-age healthy children. *S. pneumoniae* strains were serotyped and tested for antimicrobial sensitivity using disk diffusion methods. The most frequently identified serotypes were 23F, 6A, 6B, 19A, 19F, 9V, 9N and 9L. *S. pneumoniae* resistance to multiple drugs was higher in HIV-infected children (35 out of 146 isolates; 24%) compared to HIV-negative children (8 out of 78 isolates, 6.4%; RR 2.76; 95%CI 1.13;6.93).

Heptavalent pneumococcal conjugate vaccine (PCV7) has proved to be safe, effective and immunogenic in healthy children but little is known about the humoral response to this vaccine in HIV-infected children. The purpose of this study was to quantitatively evaluate humoral response to the seven pneumococcal serotypes of PVC7 vaccine in a group of 40 HIV-infected children.

METHODS

The study sample consisted of 40 HIV-infected children aged between two and nine years being followed up at a specialty outpatient clinic in São Paulo, Southeastern Brazil, between November 2002 and September 2003.

All parents or guardians of children followed up at the clinic and who met the inclusion criteria were invited to participate in the study. There was no randomization and children were included in the study following the order they attended routine visits. Inclusion criteria in the study were: age between two and nine years old; confirmed HIV infection (Brazilian Ministry of Health Decree No. 59/03); parents or guardians agreement to participate in the study. Exclusion criteria were: hypersensitivity to any of the vaccine components; thrombocytopenia; history of seizures in the 12 months prior to the study; CD4 count below 15% at the time of inclusion in the study; continuous use of intravenous immunoglobulin; no laboratory testing (CD4 count and viral load) in the three months prior to inclusion.

The vaccine studied is composed of six pneumococcal polysaccharide antigens (4, 6B, 9V, 14, 19F, 23F) and one oligosaccharide antigen (18C) conjugated with 20 µg of CRM197 protein. The recommended dose is 0.5 mL, which contains 2 µg of each antigen, except for 6B (4 µg), and 0.5 mg of aluminum phosphate added as an adjuvant.

Children underwent medical and laboratory evaluation for their inclusion in the study and then a first blood sample was collected for pre-immunization serology and the first dose of PCV7 was administered. A second dose of PCV7 was administered two months later. One month after the administration of the second dose, children underwent a second blood collection for measuring post-immunization antibody titers. In the event of blood collection delay, no more than three months were accepted between the second vaccine dose and second blood collection. Children received both doses of PCV7 intramuscularly in the deltoid muscle of the upper limb of their choice.

Serum samples were obtained before the first dose and one to three months after the second dose of PCV7. All children had their serum IgG specific against the polysaccharides of each one of the seven pneumococcal serotypes in the vaccine measured using immunoenzymatic assay (ELISA). The technique applied was described by Koskela[7] in 1987 and consisted of a single adsorption with a capsular pneumococcal polysaccharide C (Ps-C) preparation.

The estimated cutoff value for assessing antibody response was 1.3 µg/mL. Overall immunization response was considered adequate in those children with antibody titers against the pneumococcus capsule higher than or equal to 1.3 µg/mL for at least four serotypes (>50% of all serotypes). Together, other assessments at different cutoff values were also carried out: a 4-fold or more increase in post-immunization antibody titers compared to pre-immunization for four or more serotypes; and a cutoff value of 0.35 µg/mL for post-immunization titers for four or more serotypes.[17,18,20]

Pre- and post-immunization IgG antibody titers were converted into base-10 logarithm and reported as pre- and post-immunization geometric mean titers (GMT). Continuous variables were compared using paired Student's t-test. Categorical variables (response rates: ≥1.3 µg/mL and ≥4-fold increase in post- compared to pre-immunization titers according to age and prior immunization with 23-valent pneumococcal vaccine) were compared using two-tailed Fisher's exact test or chi-square test at $p<0.05$. Statistical analyses were performed using SPSS program version 10.0.

The study was approved by the Research Ethics Committee of *Instituto da Criança do Hospital das Clínicas* at Universidade de São Paulo Medical School.

RESULTS

A comparison of post- and pre-immunization GMT for each pneumococcal serotype showed that all post-immunization GMT were higher than pre-immunization GMT regardless of the serotype assessed. This difference was statistically significant ($p<0.05$) for all pneumococcal serotypes studied (Table 1).

Table 1. Distribution of geometric mean titers of pre- and post-immunization titers by pneumococcal serotype in HIV-infected children. São Paulo, Southeastern Brazil, 2002–2003. N=40

	Geometric mean titer (µg/mL)		
Serotype	Pre-	Post-	p-value
4	0.343	1.220	<0.001
6B	0.751	1.646	<0.001
9V	0.453	1.785	<0.001
14	0.935	4.525	<0.001
18C	0.509	2.045	<0.001
19F	1.513	3.097	<0.001
23F	0.517	1.482	<0.001

Table 2. Proportion of HIV-infected children showing a 4-fold increase or more in post- compared to pre-immunization antibody titers. São Paulo, Southeastern Brazil, 2002–2003. N=40

Serotype	4-fold increase or more in post-immunization antibody titers (%)
4	55.0
6B	25.0
9V	42.5
14	62.5
18C	42.5
19F	17.5
23F	30.0

Table 2 shows response rates for each vaccine serotype based on the criterion of 4-fold or more increase of post- compared to pre-immunization titers. In all samples studied, serotypes 4 (55%) and 14 (62.5%) produced the greatest increase in post-immunization IgG titers compared to pre-immunization. The poorest response was seen to serotype 19F, where only 17.5% of children had a 4-fold or more increase of IgG antibody titers.

Table 3 is based on the criterion of post-immunization IgG antibody titers equal to or greater than 1.3 µg/mL. At this cutoff, there was 50% antibody response to all serotypes studied, except serotype 4; and serotype 14 showed the highest number of children with titers equal to or greater than 1.3 µg/mL (90% of children with IgG antibody titers equal to or greater than 1.3 µg/mL in post-immunization).

After individual analysis by vaccine serotypes, adequate antibody response to the vaccine was defined as response to at least four of the seven serotypes studied.

Based on the first criterion (≥1.3 µg/mL), 26 (65%) children had antibody response to the vaccine, and 12 (30%) showed post-immunization IgG titers of at least 1.3 µg/mL for all serotypes studied. As for the serotypes studied, higher response rates were seen, in descending order, to serotypes 14 (90%), 19F (80%) and 18C (70%); and lower response rates were seen to serotypes 4 (45%) and 23F (50%).

All children showed CD4 equal to or greater than 15%, regardless of age, since this was an inclusion criterion in the study.

Based on the second criterion, 15 (37.5%) children only had ≥4-fold increase in post- compared to pre-immunization titers for four serotypes or more.

DISCUSSION

There is no established cutoff value to measure humoral response to PCV7 in individuals with HIV infection and other immunodeficiencies. Studies in healthy children[2,14] were based on different values to evaluate adequate response or the association between the response and clinical efficacy. In the present study, as there was no other option, we made use of assumptions and cutoff values described in the literature for healthy children to assess humoral response in HIV-infected children. At the time the present study was being developed, the two most remarkable criteria for evaluation were "4-fold increase or more in post-immunization titers" and "post-immunization titers ≥1.3 µg/mL". Other studies on the immunogenicity of pneumococcal conjugate vaccines in HIV-infected children have also been based on assumptions described for non-HIV-infected children while defining a protective antibody level.[4,5,13]

However, in 2005, the World Health Organization (WHO)[16,20] defined a protective antibody level against

Table 3. Proportion of HIV-infected children showing post-immunization antibody titers equal to or greater than 1.3 µg/mL. São Paulo, Southeastern Brazil, 2002–2003. N=40

Serotype	Post-immunization antibody titers ≥ 1.3 µg/mL (%)
4	45.0
6B	55.0
9V	57.5
14	90.0
18C	70.0
19F	80.0
23F	50.0

pneumococcal invasive disease for healthy children immunized with a conjugate vaccine. Humoral response to the vaccine in healthy children was defined as titers equal to or greater than 0.35 µg/mL measured using ELISA within one month post-primary immunization. During the development of the present study, we added an analysis based on this recent WHO definition.

In view of that, a meta-analysis was carried out based on three large efficacy studies: the "Kaiser Permanent of Northern California,"[2] which supported the vaccine licensing in the United States; a study with Native American populations;[14] and a South African study investigating a 9-valent conjugate vaccine also containing serotypes 1 and 5.[6]

The single protective antibody titer was defined for all vaccine serotypes assuming that antibody levels of the different serotypes were very similar. This was assumed because, even in the largest study (the Kaiser Permanente included 37,868 children),[2] the number of cases of invasive disease was still small for each specific, preventing a serotype-specific efficacy analysis.[16]

King et al[4] in 1996, defined "evidence of humoral response" as a 4-fold increase or more in post- compared to pre-immunization antibody titers. A year later,[5] while investigating the same 5-valent vaccine, they chose to set the protective antibody titer at 1,0 µg/mL based on 1981 Landesman & Schiffman study.[9]

PCV7 was first studied by Nachman et al[13] in 2003 in infants who were presumably HIV-infected. They applied the criterion of increase of antibody titers and considered responders those children who showed a 4-fold increase or more in post- compared to pre-immunization titers for three or more of the seven vaccine serotypes studied.

Madhi et al[12] in 2005, investigated a 9-valent conjugate vaccine based on the 2005 WHO[20] definitions for healthy children and established as a protective titers equal to or greater than 0.35 µg/mL.

For results interpretation in the present study, we defined adequate humoral response as those children showing post-immunization IgG antibody titers equal to or greater than 1.3 µg/mL and a 4-fold increase or more in post- compared to pre-immunization antibody titers for four or more of the seven serotypes studied. These criteria were defined by Sorensen et al,[18] in 1998, while studying healthy children and have also been applied by Marques[a] in a study of the 23-valent vaccine in AIDS children. At that time, Sorensen set the cutoff value at 1.3 µg/mL based on Lawrence et al[10] study who found that antibody titers greater than 200 ng N/mL were associated to lower nasopharynx colonization by *S. pneumoniae* in children. Lawrence's procedure was modified by Sorensen et al[18] who added the adsorption with polysaccharide C (Ps-C) to render it more adequate.

In regard to the cutoff value of 0.35 µg/mL, this antibody level is exclusively applicable for the prevention of invasive disease in immunocompetent children.[16] However, a cutoff has not been established yet for HIV-infected children, but it is believed that immunocompromised individuals may need higher antibody titers for their protection. Besides, the functional activity of IgG antibodies in HIV-infected children is lower than in healthy children.[15]

In the light of that, setting the cutoff value at 1.3 µg/mL takes into consideration the need for higher titers in HIV-infected children.

The rationale for the criterion of a 4-fold increase or more in post- compared to pre-immunization is even more complex. This would be an even more specific criterion for humoral response as it takes into account children's antibody levels before being immunized with the conjugate vaccine. Only 15 children (37.5%) had a 4-fold increase or more in post-immunization titers for at least four serotypes studied, while 26 (65%) had response at 1.3 µg/mL. Existing antibodies, from previous infections or evidenced pre-immunization, can help explain this finding. Many children in the present study were older (80% were five to nine years old) and likely to have been naturally exposed to different serotypes of *S. pneumoniae* and 22 children (59.5%) had previously been immunized with a 23-valent polysaccharide vaccine. These factors may have affected humoral response measured in the present study.

Natural pneumococcal exposure is believed to "prime" the immune system. The polysaccharide capsule of the outer bacterial membrane proteins may be identified by immune cells as T-dependent antigens, eliciting a response with the production of memory cells. This immune memory becomes evident when individuals are secondarily exposed to a polysaccharide antigen and their body reacts with rapid, intense antibody production (anamnestic response). As mentioned before, this is the type of response elicited by the conjugate vaccine.[19]

Data on the kinetics of the immune memory in immunocompromised individuals are scarce. Rose et al[15] investigated 33 children with history of repeat respiratory infections. They were divided into two groups: Group A received two doses of pneumococcal conjugate vaccine and a year later a dose of 23-valent polysaccharide vaccine; while Group B received two doses only of polysaccharide vaccine administered one year apart. Group A, that has been previously immunized with a

[a] Marques, HHS. Qualitative and quantitative antibody evaluation in human immunodefficiency (HIV) infected children [doctorate thesis]. São Paulo: USP Medical School; 2000.

conjugate vaccine, showed stronger and very rapidly response with higher antibody titers after exposure to the polysaccharide vaccine (on Day 7 post-immunization most subjects showed antibody titers ≥1.0 µg/mL for all serotypes in the 7-valent vaccine).

Compared to conjugate vaccines, polysaccharide vaccines do not have the same power. Its T-independent immune response does not lead to immune system "priming," and the production of memory cells, resulting in rapid reduction of antibody levels over time. In the present study, 59.5% of children had previously been immunized with the 23-valent polysaccharide vaccine.

The scarcity of studies on pneumococcal conjugate vaccine in HIV-infected children allied to different sample selection and criteria of humoral response prevent any comparison of specific humoral response by vaccine serotype found in the present study with other studies in the literature. For both criteria applied in the present study (post-immunization titers ≥1.3 µg/mL and a 4-fold increase or more in post-compared to pre-immunization titers), the best humoral response was seen to serotype 14 and the worst response was seen to serotypes 6B and 23F. Regardless of the criterion of humoral response applied, the finding of adequate response to serotype 14 and poor response to 6B is corroborated in the literature. In all studies reviewed, serotype 14 always elicited the highest titers post-immunization with the conjugate vaccine.[5,12,13] Serotypes 9V and 19F also seem to elicit strong responses, while 18C and 23F seem to elicit weak responses.[5,12,13]

In regard to HIV-related immune compromising in the children of the study, CD4 was used as a marker and measured within three months prior to immunization. Although 27.5% of the children studied were classified as category C, 95% were receiving antiretroviral therapy and had CD4 ≥25%. Due to this immune recovery, many children who previously had severe symptoms and opportunistic infections (classified as C), were at the time of inclusion in the study asymptomatic or showed mild signs and symptoms. In contrast, only two of them showed CD4 counts between 15% and 25%, preventing an analysis of the effect of these counts on humoral response of this subgroup separately.

With respect to the response to four or more serotypes, 27 (67.5%) children had humoral response with titers of 1.3 µg/mL or more, which can be considered adequate in people with immunodeficiencies that may affect this response.

Based on the criterion of a 4-fold increase or more in post- compared to pre-immunization titers for four or more serotypes, response was seen in only 15 (37.5%) children, which is lower than that found when the first criterion was applied.

In the light of recent studies and mostly recent WHO[20] definitions of protective levels for invasive disease in healthy children, we chose to complete data analysis with an evaluation at a cutoff value of 0.35 µg/mL. Only Madhi et al[12] have applied WHO criteria[21] in HIV-infected children. The authors have measured antibodies to nine serotypes of the vaccine studied and found more than 90% response to six or more serotypes, which corroborates the results in 38 children (95%) of the present study.

Since different criteria produced different results, we concluded that further studies are needed in HIV-infected children to better describe the actual immune response of children to PCV7.

REFERENCES

1. Bernstein LJ, Krieger BZ, Novick B, Sicklick MJ, Rubinstein A. Bacterial infection in the acquired immunodeficiency syndrome of children. *Pediatr Infect Dis J*. 1985;4(5):472-5. DOI: 10.1097/00006454-198509000-00005

2. Black S, Shinefield H, Fireman B, Lewis E, Ray P, Hansen, JR, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. *Pediatr Infect Dis J*. 2000;19(3):187-95. DOI: 10.1097/00006454-200003000-00003

3. Farley JJ, King JC Jr, Nair P, Hines SE, Tressler RL, Vink PE. Invasive pneumococcal disease among infected and uninfected children of mothers with human immunodeficiency virus infection. *J Pediatr*. 1994;124(6):853-8. DOI: 10.1016/S0022-3476(05)83170-1

4. King JC Jr, Vink PE, Chang IH, Kimura A, Parks M, Smilie M, et al. Antibody titers eight months after three doses of a five-valent pneumococcal conjugate vaccine in HIV and non-HIV-infected children less than two years of age. *Vaccine*. 1996;16(4):361-5. DOI: 10.1016/S0264-410X(97)80914-0

5. King JC Jr, Vink PE, Farley JJ, Smilie M, Parks M, Lichenstein R. Safety and Immunogenicity of three doses of a five-valent pneumococcal conjugate vaccine in children younger than two years with and without human immunodeficiency virus infection. *Pediatrics*. 1997;99(4):575-80. DOI: 10.1542/peds.99.4.575

6. Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N, Pierce N. A trial of a 9-valent pneumococccal conjugate vaccine in children with and those without HIV infection. *N Engl J Med*. 2003;349(14):1341-8. DOI: 10.1056/NEJMoa035060

7. Koskela M. Serum antibodies to pneumococcal C polysaccharide in children: response to acute pneumococcal otitis media or to vaccination. *Pediatr Infect Dis J*. 1987;6(6):519-26. DOI: 10.1097/00006454-198706000-00006

8. Krasinski K, Borkowsky W, Bonk S, Lawrence R, Chandwani S. Bacterial infections in human immunodeficiency virus-infected children. *Pediatr Infect Dis J*. 1988;7(5):323-8. DOI: 10.1097/00006454-198805000-00006

9. Landesman SH, Schiffman G. Assessment of the antibody response to pneumococccal vaccine in high risk population. *Rev Infect Dis*. 1981;3(Suppl):S184-97.

10. Lawrence EM, Edwards KM, Schiffman G, Thompson JM, Vaughan WK, Wright PF. Pneumococcal vaccine in normal children. *Am J Dis Child*. 1983;137(9):846-50.

11. Madhi SA, Petersen K, Madhi A, Wasas A, Klugman KP. Impact of human immunodeficiency virus type 1 on the disease spectrum of *Streptococcus pneumoniae* in south african children. *Pediatr Infect Dis J*. 2000;19(12):1141-7. DOI: 10.1097/00006454-200012000-00004

12. Madhi SA, Kuwanda L, Cutland C, Holm A, Käyhty H, Klugman KP. Quantitative and qualitative antibody response to pneumococcal conjugate vaccine among african human immunodeficiency virus-infected and uninfected children. *Pediatr Infect Dis J*. 2005;24(5):410-6. DOI: 10.1097/01.inf.0000160942.84169.14

13. Nachman S, Kim S, King J, Abrams EJ, Margolis D, Petru A, et al. Safety and immunogenicity of a heptavalent pneumococcal conjugate vaccine in infants with human immunodeficiency virus type 1 infection. *Pediatrics*. 2003;112(1 Pt 1):66-73. DOI: 10.1542/peds.112.1.66

14. O'Brien KL, Moulton LH, Reid R, Weatherholtz R, Oski J, Brown L, et al. Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomized trial. *Lancet*. 2003;362(9381):355-61. DOI: 10.1016/S0140-6736(03)14022-6

15. Rose MA, Schubert R, Strand N, Zielen S. Priming of immunological memory by pneumococcal conjugate vaccine in children unresponsive to 23-valent polysaccharide pneumococcal vaccine. *Clin Diagn Lab Immunol*. 2005;12(1):1216-22. DOI: 10.1128/CDLI.12.10.1216-1222.2005

16. Siber GR, Chang I, Baker S, Fernsten P, O'Brien KL, Santosham M, et al.. Estimating the protective concentration of anti-pneumococcal capsula poltsaccharide antibodies. *Vaccine*. 2007;25(19):3816-26. DOI: 10.1016/j.vaccine.2007.01.119

17. Sorensen RU, Hidalgo H, Moore C, Leiva LE. Post-immunization pneumococcal antibody titers and IgG subclasses. *Pediatr Pneumol*. 1996;22(3):167-73.

18. Sorensen RU, Leiva LE, Giangrosso PA, Butler B, Javier, FC 3rd, Sacerdote DM, et al. Response to a heptavalent conjugate *Streptococcus pneumoniae* vaccine in children with recurrent infections who are unresponsive to the polysaccharide vaccine. *Pediatr Infect Dis J*. 1998;17(8):685-91. DOI: 10.1097/00006454-199808000-00005

19. Spoulou VI, Tsoumas DL, Papaevangelou VG, Mostrou GI, Theodoridou MC. Immunogenicity and immunological memory induced by a 7-valent pneumococcal CRM197 conjugate vaccine in symptomatic HIV-1 infected children. *Vaccine*. 2005;23(46-47):5289-93. DOI: 10.1016/j.vaccine.2005.06.002

20. World Health Organization. Recommendations for the production and control of pneumococcal conjugate vaccines. Geneva; 2005. (WHO Technical Report Series, 927).

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