Immunogenicity and safety of pneumococcal conjugate polysaccharide and free polysaccharide vaccines alone or combined in HIV-infected adults in Brazil

Yeh-Li Ho, Angela Pires Brandão, Maria Cristina de Cunto Brandileone, Marta Heloisa Lopes

Article history:
Received 13 February 2013
Received in revised form 5 April 2013
Accepted 24 April 2013
Available online 16 May 2013

ABSTRACT

Background: Streptococcus pneumoniae is a leading cause of hospitalization in HIV-infected adults; therefore pneumococcal vaccine is recommended. The ideal antipneumococcal vaccine and effective vaccination regimen remain controversial and need further evaluation.

Methods: To assess the efficacy of pneumococcal vaccines alone and combined, a randomized, blinded clinical trial was conducted in Brazil with 331 HIV-infected patients aged 18–60, with CD4-T cell count ≥ 200 cells/mm3. Two interventions 60 days apart were done in three schedules: 23-valent pneumococcal polysaccharide vaccine (PPV23)/placebo; 7-valent pneumococcal conjugate vaccine (PCV7)/placebo; and PCV7 plus PPV23. Safety and reactogenicity were evaluated, and immunogenicity was assessed by an IgG enzyme-linked immunosorbent assay to S. pneumoniae serotypes 6B, 9V and 14, performed at baseline, 60 and 180 days after first intervention. Comparison of immunogenicity was based on geometric mean concentration (GMC), percentages of individuals with serotype-specific IgG ≥ 0.35 μg/mL and ≥ 1.0 μg/mL and proportion of individuals with ≥4-fold increase in specific antibody concentrations for each serotype.

Results: Demographic and HIV conditions were similar, and both vaccines were well tolerated across vaccine groups. Significant increase in IgG-antibodies was observed for all serotypes evaluated. A greater proportion of PCV7 recipients reached and sustained IgG antibody concentrations at least four times as high as those at baseline, for serotypes 6B and 9V. A PPV23 dose after PCV7 did not enhance immunogenicity.

Conclusions: In this first trial conducted with HIV-infected immunologically stable adults in South America, both PPV23 and PCV7 were safe and immunogenic. Evidence suggesting PCV7 was more immunogenic than PPV23, as it elicited higher and persistent ≥4-fold increase of antibodies for 6B and 9V serotypes in a greater proportion of HIV-patients is noteworthy. Despite current recommendation of schedules combining PCV7 and PPV23, there is little evidence to support this practice and we did not observe benefits in this combination.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Invasive pneumococcal disease (IPD), as other bacterial diseases and sepsis represent important causes of hospitalization in human immunodeficiency virus (HIV) patients in the era of the highly active antiretroviral treatment (HAART) [1,2]. The risk of IPD in HIV-infected persons is 23–60 times higher than in HIV-uninfected individuals [3,4], even in the HAART era [5], and the mortality is also higher for HIV-infected adults [3,4]. Therefore, strategies to reduce the burden of IPD are crucial.

The 23-valent pneumococcal polysaccharide vaccine (PPV23), although recommended for HIV-infected adults, presents lower immunogenicity and effectiveness than to HIV-uninfected persons. Clinical trials showed impaired antibody response to PPV23 in HIV-infected patients compared to healthy individuals [6]. Additionally, antibody responses may be CD4-T cell dependent [7,8], making PPV23 administration preferential in the early course of HIV infection, when CD4-T cell counts are ≥ 200 cells/mm3 [9], or after immune restoration through HAART. According to a meta-analysis [10], PPV23 was not effective in reducing IPD-related mortality rates in adults, even in populations for whom PPV23 is currently
recommended, despite vaccine protection for presumptive pneumococcal pneumonia and for all-cause pneumonia. In HIV-infected adults, conflicting results about PPV23 efficacy against IPD, pneumococcal pneumonia and other bacterial pneumonia have also been observed [11–18].

The use of pneumococcal conjugate vaccine (PCV) in adults is still controversial [19,20]. Some clinical trials in nonimmunosuppressed adults revealed higher antibody responses to PCV than to PPV23 [21–23], while inferior antibody response to PCV in elderly individuals was observed [24]. In a clinical trial comparing PPV23 and PCV in HIV-infected adults [25], higher antibody responses were observed after PCV for serotypes 6B, 18C, and 23F, but not for 19F. In HIV-infected adults with CD4-T cells counts ≥200 cells/mm³, no difference was found between PPV23 and PCV for serotypes 4, 6B, 9V, 14, and 23F [26]. In one of the few studies evaluating the effectiveness of PCV in HIV-infected adults [27], significant protection against recurrence of IPD in HIV-infected adults was observed, however, this protection has decreased dramatically over time, from 85% in the first year to 25% thereafter.

Combined PCV/PPV23 schedules have been tested for HIV-infected adults. Two PCV doses given a month apart, followed by PPV23 nine months later, elicited significant increment of antibody concentration [28]. Lesprit et al. [29] observed in HIV-adults with CD4-T cell counts of 200–500 cells/mm³ higher immunological response to PCV followed by PPV23 compared to one dose of PPV23, while no difference was observed between two doses of PCV and PCV/PPV23 schedules. On the other hand, no antibody increase was seen in a study [26] using PCV followed by either conjugate or polysaccharide vaccine.

This study aims to compare antibody response to PPV23 and PCV7 alone and combined in HIV-infected adults.

2. Materials and methods

2.1. Study design

A randomized, blinded clinical trial to compare the immunogenicity of PPV23 and PCV7 given in different schedules to HIV-infected adults was performed. Patients received two vaccine doses given 60 days apart, according to three vaccination schedules, as follows: PPV23 and then placebo vaccine (group A); PCV7 followed by placebo vaccine (group B); and PCV7 followed by PPV23 (group C). To assess vaccine-induced immune responses, peripheral venous blood samples were collected just before each vaccine dose (at baseline and day 60), and on day 180 (120 days after second dose). Simultaneously, blood samples were collected to perform CD4-T cells count and HIV viral load tests.

Sample size calculations, with a significance level of 0.05, a power of 0.80, and 10% loss to follow-up resulted in 140 patients in each group. Allocation to groups was on 1:1:1 basis generated by a table of random numbers from Epi-info version 6.04. Study investigators and laboratorial executors were blinded to group allocation.

2.2. Study population

HIV-infected outpatients were recruited at the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, Brazil, the largest public hospital in South America. Individuals aged 18–60, with HIV infection documented by enzyme-linked immunosorbsent assay (ELISA) and Western-blott testing, and with CD4-T cell count ≥200 cells/mm³ in two different occasions in the past six months were eligible. Exclusion criteria included: any acute febrile illness at the moment of vaccination, active AIDS-defining clinical condition, previous immunization with any of the pneumococcal vaccines, any systemic malignancy neoplasm, use of immunoglobulin within the last three months, current pregnancy and antecedent of allergy to any of the pneumococcal vaccine components.

The study was approved by the Ethics Committee at HCFMUSP (process number 047/04). The trial is registered in clinical trial registry/database under the number RBR-3P7577. Written informed consent was obtained from all participants. Principles of Declaration of Helsinki were followed and the study was conducted adhering to good clinical practice guidelines.

After screening, individual interview was performed and medical record was reviewed to obtain demographic characteristics and information related to HIV infection.

2.3. Vaccine administration

PPV23 (Pneumovax; Aventis Pasteur) contained 25 μg/dose of Streptococcus pneumoniae serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F. PCV7 (Prevenar; Wyeth) contained per dose 2 μg of pneumococcal serotypes 4, 9V, 14, 18C, 19F, 23F and 4 μg of serotype 6B individually conjugated to diphtheria CRM197 protein. Placebo vaccine consisted of physiological saline solution. Doses of 0.5 mL were administered by research nurses as deltoid-intramuscular injections.

2.4. Immunogenicity

Pneumococcal serotypes 6B, 9V, and 14 were selected for immunogenicity studies based on IPD data in adults [30] and on pneumococcal carriage by HIV-infected adults living in São Paulo [31] at the time this study was designed. They were among the most frequently isolated both from IPD and carriage and are common to both vaccines.

Total IgG anticapsular antibody concentrations to serotypes 6B, 9V, and 14 were measured by ELISA, according to the World Health Organization protocol [32]. In order to avoid assay variability, same lot microtiter plates, capsular and C-polysaccharides, and enzyme-conjugated anti-human IgG were used throughout this study. Before the analysis, both optimal antigen coating concentration and enzyme-conjugated anti-human IgG dilution were determined. Serum samples, aliquoted and stored at −70 °C until assayed, were reacted with pneumococcal C-polysaccharide and 22F capsular polysaccharide to adsorb antibodies to common contaminants that might be present in the coating antigens. Every ELISA plate contained a pooled human serum sample from vaccinated individuals as quality control and eight duplicate dilutions of a calibration standard, 89-SF (not adsorbed with serotype 22F polysaccharide). All serum samples belonging to a patient were analyzed simultaneously on the same plate, in duplicate and in six serial dilutions.

Antibody concentrations were calculated by comparing test sera with 89-SF using a four-parameter curve fit program from ELISA software [33]. Results were expressed as μg/mL calculated on the basis of the assigned IgG values for 89-S reference serum [34,35]. Samples with antibody concentrations <0.1 μg/mL or >100 μg/mL were confirmed upon retesting.

2.5. Statistical analysis

The pre- and post-vaccine antibody concentrations against each serotype were log transformed to obtain normality, and the results expressed as geometric mean concentration (GMC).

The following parameters were calculated: (a) GMCs for serotypes 6B, 9V and 14; (b) the percentages of individuals with serotype-specific IgG ≥0.35 μg/mL and ≥1.0 μg/mL; (c) proportion
of individuals with ≥4-fold increase in antibody concentrations for each serotype.

The values of 0.35 µg/mL and 1.0 µg/mL chosen as cut-off points do not necessarily correspond to seroprotective levels. Despite 0.35 µg/mL has been defined as the correlate of protection against IPD in infant, no seroprotective level has yet been established for adults.

Microsoft Excel 2003 was used to enter all data and statistical analysis was performed using Epi-Info v3.5 and StatPlus Professional v5.8.4. Differences between groups in proportion of individuals with IgG ≥0.35 µg/mL, IgG ≥1.0 µg/mL, and ≥4-fold increase in antibody concentrations were determined by two-tailed Fisher exact and Pearson Chi-squared tests. Medians were compared using ANOVA or Kruskal–Wallis tests. The level of significance was set at \( p \leq 0.05 \), with 95% confidence intervals (CI).

### 3. Results

#### 3.1. Study population

From October 2005 to May 2009, 331 patients were enrolled in the study (111 individuals to group A, 110 to group B and 110 to group C). Enrollment was interrupted because institution overloading prevented further admissions of recently diagnosed HIV-patients. There was no differential loss to follow-up among study groups (Fig. 1).

Mean age of patients was 40.6 years, 74.6% male, mean time of HIV diagnosis was 6.7 years, and 60.1% had previous AIDS-defining clinical condition. Mean of CD4-T cells count was 525 cells/mm\(^3\) and 67.3% had HIV-viral load <400 copies/mm\(^3\). HAART was in use by 76.6% of patients. The three groups were similar in demographic characteristics and HIV conditions (Table 1).

#### 3.2. Immunogenicity

At baseline there were no significant differences among the three groups in GMCs of antipneumococcal antibodies or in the proportion of individuals with IgG antibody concentration ≥0.35 µg/mL or ≥1.0 µg/mL for all serotypes analyzed (Table 2).

Sixty days after the initial dose of either PPV23 (group A) or PCV7 (groups B and C), all study groups showed significant increases in GMCs of the antipneumococcal antibodies for all serotypes (Fig. 2). At day 180, the antibody levels decreased in all groups for all serotypes, though less markedly for serotypes 6B and 14 in recipients of a PCV7 dose and PPV23 booster 60 days later (group C) (Fig. 2).

The mean concentration of antipneumococcal antibodies and the proportion of individuals with antibody concentrations ≥0.35 µg/mL and ≥1.0 µg/mL 60 and 180 days post-vaccination were similar for the three serotypes analyzed. However, the proportion of individuals who achieved at least a 4-fold increase in antibody levels was higher to serotypes 6B and 9V in patients primed with PCV7 (groups B and C), in both 60 and 180 days (Table 2).

No statistical differences were observed after receipt of PCV7 alone (group B) compared with a PPV23 dose 60 days after PCV7 priming (group C) as measured by antipneumococcal antibody GMCs, proportion of individuals with IgG antibody concentrations ≥0.35 µg/mL or ≥1.0 µg/mL, or presenting ≥4-fold increases in antibody levels (Table 2).

#### 3.3. Reactogenicity

All strategies were well tolerated. No severe local or systemic symptoms were reported. After first vaccination, no statistical difference was observed between PPV23 and PCV7 in terms of local reaction, as pain, redness and swelling at the injection site. However, higher proportion of individuals vaccinated with PCV7 reported systemic symptoms, including fever, myalgia and asthenia (Table 3). No statistical difference was observed between PPV23 given alone (group A) or as a booster dose (group C).

### 4. Discussion

The role of pneumococcal vaccination and the best antipneumococcal vaccine to be recommended in adults is still controversial [19,20]. Recently, a pneumococcal 13-valent conjugate vaccine (PCV13) was approved in the USA for people aged ≥50 years and the Advisory Committee on Immunization Practices of USA (ACIP) recommended routine use of PCV13 for adults aged ≥19 years with immunocompromising conditions [36]. However, many questions about the use of pneumococcal vaccines are still unclear [37]. Furthermore, different from infants, the protective specific antibody concentration in adults is not determined. Questions as the best condition and the ideal schedule of vaccination of HIV-infected adults, and the lower post-vaccination responses than by immunocompetent individuals are otherwise unresolved problems [38–41].

Despite only patients with CD4-T cell ≥200 cells/mm\(^3\) have been enrolled, some studies observed no impact of CD4-T count on PCV23 immunogenicity and determined the potential influence of antiretroviral therapy and HIV-viral load at the moment of vaccination with protective effect [14–16]. In our trial, the majority of patients was in current antiretroviral therapy and had HIV-viral load <400 copies/mm\(^3\).

Antibodies levels at baseline were high to the three serotypes, especially to serotype 14, what might in part explain the absence of significant differences after vaccination, in terms of frequency of individuals with antibody concentrations ≥0.35 µg/mL or ≥1.0 µg/mL. Therefore, analysis of immunogenicity based on these two parameters may be of little value for some populations and age groups unlikely to be naïve to pneumococcal antigens.

The finding that the proportion of individuals who reached a ≥4-fold increase in antibodies levels 60 days from baseline was higher in recipients of PCV7 may imply superior immunogenicity

### Table 1

Baseline characteristics of the study population at time of inclusion.

<table>
<thead>
<tr>
<th></th>
<th>Group A (N=111)</th>
<th>Group B (N=110)</th>
<th>Group C (N=110)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)(^a)</td>
<td>77 (69.5)</td>
<td>86 (78.2)</td>
<td>84 (76.4)</td>
<td>0.28</td>
</tr>
<tr>
<td>Age, mean ± SD(^b)</td>
<td>40.16 ± 9.41</td>
<td>40.12 ± 8.51</td>
<td>41.55 ± 7.76</td>
<td>0.37</td>
</tr>
<tr>
<td>AIDS, n (%)(^a)</td>
<td>65 (58.6)</td>
<td>65 (59.1)</td>
<td>69 (62.7)</td>
<td>0.79</td>
</tr>
<tr>
<td>CD4-T cells, mean ± SD(^a)</td>
<td>548 ± 304</td>
<td>545 ± 238</td>
<td>492 ± 269</td>
<td>0.07</td>
</tr>
<tr>
<td>HIV viral load &lt;400 copies/mm(^3), n (%)(^a)</td>
<td>71 (64)</td>
<td>74 (67.3)</td>
<td>79 (72.5)</td>
<td>0.39</td>
</tr>
<tr>
<td>HAART, n (%)(^a)</td>
<td>81 (73)</td>
<td>84 (76.4)</td>
<td>89 (80.9)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

SD, standard deviation; AIDS, acquired immunodeficiency syndrome–defining clinical condition; HAART, highly active antiretroviral therapy.

\(^a\) Chi-squared test.

\(^b\) ANOVA.

\(^a\) Kruskal–Wallis.
## Table 2
Serum IgG antibodies responses to pneumococcal capsular polysaccharides serotypes 6B, 9V, and 14 from HIV-infected adults before and after pneumococcal vaccines given in three vaccine schedules.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Serum sample</th>
<th>Mean IgG concentration*, μg/mL (G&lt;sub&gt;60&lt;/sub&gt;)</th>
<th>IgG concentration&lt;sup&gt;a&lt;/sup&gt; ≥0.35 μg/mL, N(%)</th>
<th>IgG concentration&lt;sup&gt;a&lt;/sup&gt; ≥1.0 μg/mL, N(%)</th>
<th>IgG fold increase&lt;sup&gt;a&lt;/sup&gt; ≥4, N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>6B</td>
<td>Pre&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73 (0.56–0.94)</td>
<td>0.51 (0.39–0.65)</td>
<td>0.57 (0.44–0.74)</td>
<td>76(68.5)</td>
</tr>
<tr>
<td>60 days</td>
<td></td>
<td>1.45&lt;sup&gt;c&lt;/sup&gt; (1.04–2.01)</td>
<td>1.88&lt;sup&gt;c&lt;/sup&gt; (1.35–2.64)</td>
<td>2.12&lt;sup&gt;c&lt;/sup&gt; (1.49–3.01)</td>
<td>74(74.0)</td>
</tr>
<tr>
<td>180 days</td>
<td></td>
<td>1.02&lt;sup&gt;d&lt;/sup&gt; (0.73–1.42)</td>
<td>1.24&lt;sup&gt;d&lt;/sup&gt; (0.88–2.50)</td>
<td>1.76&lt;sup&gt;d&lt;/sup&gt; (1.24–2.5)</td>
<td>67(75.3)</td>
</tr>
<tr>
<td>9V</td>
<td>Pre</td>
<td>0.55 (0.43–0.69)</td>
<td>0.43 (0.33–0.55)</td>
<td>0.47 (0.38–0.58)</td>
<td>75 (67.9)</td>
</tr>
<tr>
<td>60 days</td>
<td></td>
<td>1.76&lt;sup&gt;e&lt;/sup&gt; (1.34–2.31)</td>
<td>2.74&lt;sup&gt;e&lt;/sup&gt; (2.00–3.77)</td>
<td>2.97&lt;sup&gt;e&lt;/sup&gt; (2.18–4.03)</td>
<td>90 (90.0)</td>
</tr>
<tr>
<td>180 days</td>
<td></td>
<td>1.37&lt;sup&gt;f&lt;/sup&gt; (1.03–1.82)</td>
<td>1.68&lt;sup&gt;f&lt;/sup&gt; (1.22–2.31)</td>
<td>2.13&lt;sup&gt;f&lt;/sup&gt; (1.58–2.88)</td>
<td>79 (88.8)</td>
</tr>
<tr>
<td>14</td>
<td>Pre</td>
<td>1.82 (1.36–2.42)</td>
<td>1.15 (1.08–2.16)</td>
<td>1.40 (1.01–1.91)</td>
<td>95 (85.6)</td>
</tr>
<tr>
<td>60 days</td>
<td></td>
<td>8.94&lt;sup&gt;g&lt;/sup&gt; (6.36–12.58)</td>
<td>12.36&lt;sup&gt;g&lt;/sup&gt; (8.15–18.74)</td>
<td>8.01&lt;sup&gt;g&lt;/sup&gt; (5.70–11.26)</td>
<td>96 (96.0)</td>
</tr>
<tr>
<td>180 days</td>
<td></td>
<td>6.72&lt;sup&gt;h&lt;/sup&gt; (4.65–9.72)</td>
<td>9.32&lt;sup&gt;h&lt;/sup&gt; (6.04–14.38)</td>
<td>6.35&lt;sup&gt;h&lt;/sup&gt; (4.36–9.26)</td>
<td>86 (96.6)</td>
</tr>
</tbody>
</table>

CI denotes confidence interval.
A denotes group A, comprising persons vaccinated with 23-valent pneumococcal polysaccharide vaccine (PPV23) and placebo 60 days apart.
B denotes group B, vaccinated with pneumococcal 7-valent conjugate vaccine (PCV7) and placebo 60 days apart.
C denotes group C, in which persons were vaccinated with PCV7 followed by PPV23 60 days apart; n/a denotes not applicable.

Differences in means of IgG investigated by analysis of variance; else, by Pearson’s Chi-square.

<sup>a</sup> Concentrations calculated in geometric mean.
<sup>b</sup> Pre-vaccination, N = 111 (group A), 109 (group B), 1 sample accidentally lost, 110 (group C).
<sup>c</sup> 60 days after 1st vaccine, N = 100 (groups A and B, 2 samples accidentally lost), 105 (group C).
<sup>d</sup> 180 days after 1st vaccine, N = 89 (group A), 91 (group B), 91 (group C, 1 sample accidentally lost).
<sup>e</sup> Pre < 0.05 for pre-vaccination vs 60 days.
<sup>f</sup> p < 0.05 for pre-vaccination vs 180 days.
<sup>“</sup> p < 0.001 for group A vs groups B and C.
induced by a single dose of PCV7 compared with PPV23. Our finding is in contrast with a recent study [42] using a different clinical trial design, in which 2-fold increases in antibody concentration between PCV7 and PPV23 were similar.

Decline in antibody concentrations 180 days after initial dose was similar and expectedly slight for all vaccine schedules. Interestingly again was the significant larger proportion of PCV7 recipients keeping higher serotypes 6B and 9V capsular antibody increments than those primed with PPV23. Consistent with previous reports [26,42] but in contrast with another study [29], we observed no benefit of booster with PPV23 after priming with PCV7.

Similar to previous studies [26,28,29], both vaccines were well tolerated. Systemic symptoms were more frequent after receipt of PCV7 than of PPV23, as observed in prior study [42], but in contrast with other [26]. Previous exposition to PCV7 did not alter the reactogenicity to PPV23, as reported earlier [28].

Limitations of our study comprise the narrow subset of vaccine serotypes evaluated, though they represent the most
common serotypes causing IPD in adults in Brazil [30]. Evaluating vaccine-induced antibodies to more serotypes would add further information on the immunogenicity elicited by different vaccine schedules. Functional antibody activity, as measured by opsonophagocytic assay, was not assessed in our trial, however, previous studies have demonstrated that adsorbing test sera with pneumococcal 23F capsular polysaccharide improves correlation of IgG-ELISA with opsonophagocytic activity [43–45].

A major problem in immunogenicity trials in adults is the lack of a defined level of antibodies that correlates with disease prevention. Although IgG antibody levels ≥1.0 μg/mL for the majority of serotypes is required for protection in children [46], this is indefinite in adults, and the best method to determine post-vaccine serological turning is still controversial.

In conclusion, this first pneumococcal vaccine trial in HIV-infected adults immunologically stable conducted in South America showed both PPV23 and PCV7 elicited significant increase of IgG antibodies to serotypes 6B, 9V, and 14. PCV7 provided higher proportion of individuals who reached 4-fold increase of IgG and sustained this condition to serotypes 6B and 9V. Despite current recommendation of schedules combining PCV7 and PPV23 [38], there is little evidence to support this practice and we did not observe benefit in this combination. Both vaccines were well tolerated in HIV-infected adults.

Acknowledgments

This trial has received financial support from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), process number 2005/01414-7. The authors are thankful to all study subjects that participated in this clinical trial. We also appreciate the work of nurses and staff of HIV-infected Outpatient Center of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. Special thanks to Camila Fonseca Rizek for her help in laboratory assays. Special acknowledgment to Dr. David Goldblatt who permitted the first author of this trial to training in ELISA performed at the WHO Reference Laboratory for Pneumococcal Serology based at the UCLA Institute of Child Health in London.

References


