A Randomized Study Comparing Combined Pneumococcal Conjugate and Polysaccharide Vaccination Schedules in Adults

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Background. The widely used 23-valent plain polysaccharide vaccine (23vP) has limited effectiveness, produces short-lived immune responses, and induces attenuated antibody production after subsequent challenge with pneumococcal vaccines. Our goal was to examine whether priming with the 7-valent pneumococcal conjugate vaccine (PCV7) could enhance the immunogenicity of 23vP for the PCV7 serotypes and to investigate whether 23vP induced hyporesponsiveness could be overcome using PCV7.

Methods. We conducted an open-label randomized study that compared 3 vaccine schedules, each of which consisted of 2 doses of PCV7 and 1 dose of 23vP (23vP-PCV7-PCV7, PCV7-23vP-PCV7, PCV7-PCV7-23vP) administered over a 1-year period in a cohort of 348 adults 50–70 years of age. All vaccines were administered intramuscularly and were given 6 months apart. Blood samples were obtained prior to and 1 month after each vaccination.

Results. 23vP administered after priming with 2 doses of PCV7 produced significantly higher antibody concentrations for 3 of the 7 PCV7 serotypes, compared with vaccination with a single dose of 23vP; however, the same immunogenicity could be achieved with a single dose of PCV7. Prior vaccination with 23vP attenuated the antibody response to subsequent PCV7, which was not restored by additional doses of PCV7.

Conclusion. In adults, vaccination schedules combining PCV7 and 23vP do not provide improved immunogenicity over the use of a single dose of 23vP for most of the serotypes contained in PCV7.

Pneumococcal infections disproportionately affect the elderly population [1]. The associated mortality can be as high as 37% in those >80 years of age [2]. Accordingly, a 23-valent plain polysaccharide plain vaccine (23vP) is recommended for those >65 years of age in the United States and the United Kingdom. Meta-analyses show that 23vP provides 50% protection against invasive pneumococcal disease, but it affords no protection against pneumonia or death from invasive pneumococcal disease [3]. 23vP induces robust antibody levels in elderly individuals, so the reasons for its poor effectiveness are not yet established.

Vaccination schedules that combine both 23vP and new pneumococcal conjugate vaccines (PCVs) are an attractive proposition, because they may maintain the breadth of serotype coverage afforded by 23vP and also provide the potential immunological advantages afforded by PCVs for some serotypes. Conjugation of a plain polysaccharide to a carrier protein transforms the immune response to a T-dependent (TD) response, which has the advantage of generating higher-affinity antibodies, immunological memory, and responsiveness to booster doses of vaccine. In contrast, plain polysaccharide vaccines do not generate immunological memory, and repeated doses do not elicit booster responses. Moreover, previous studies have shown that 23vP induces attenuated or hyporesponsive antibody responses to...
subsequent vaccination with plain or conjugated pneumococcal vaccines [4–7].

This study was undertaken to assess the potential of vaccination schedules that combine 23vP and PCV7 for use in adults >65 years of age to improve immunogenicity in this age group. This study was registered with the International Standard Randomised Controlled Trial Number (ISRCTN) database (ISRCTN: 78768849).

**METHODS**

**Participants and Recruitment**

A phase-4, open-label, randomized, parallel trial was conducted in Oxford, UK, involving adults 50–70 years of age. Exclusion criteria were as follows: previous anaphylactic reaction to a vaccine component, previous pneumococcal vaccination or disease, receipt of diphtheria toxoid or CRM197-containing vaccine within the past year, immune dysfunction, receipt of blood products within the past 3 months, and prolonged bleeding time. Determination of previous pneumococcal vaccination was based on the information provided by the participant but was confirmed after enrollment by contacting the participant’s general practitioner. Although routine pneumococcal vaccination is aimed at adults >65 years of age, the age range of participants included in this study was 50–70 years to recruit a sufficient number of participants who had not previously received 23vP. Written informed consent was obtained from the participants before enrollment. Ethical approval was obtained from the Oxfordshire Research Ethics Committee 06/Q1604/121.

**Study Objectives**

The primary objective of the study was the antibody response to 23vP after priming with 0, 1, or 2 doses of PCV7 for the 7 serotypes contained in PCV7. The secondary objectives were to assess the effect of prior vaccination with 23vP on antibody responses to a single dose of PCV7 and to assess the recovery of antibody responses after 2 sequential doses of PCV7. Other secondary objectives, which will be reported separately when result are finalized, were to investigate memory B cell responses to PCV7 before or after 23vP, the relationship between memory B cell frequency and antibody responses, the phenotype of the B cell response involved, the reactogenicity of the vaccine schedules, and genetic polymorphisms and their effect on the immune response to vaccination.

**Study Design**

Participants were randomized to receive 1 of 3 vaccination schedules: 23vP followed by 2 doses of PCV7 (group 1), with no priming with PCV7 prior to 23vP administration; PCV7-23vP-PCV7 (group 2), with 1-dose priming with PCV7 prior to 23vP administration; or PCV7-PCV7-23vP (group 3), with 2-dose priming with PCV7 prior to 23vP administration. The randomization list was produced by the study statistician. Allocation to groups was on a 1:1:1 basis generated by a computerrandomization scheme that incorporated stratification by age and sex. Randomization began with a block size of 7 for each stratum in the first instance and then employed various block sizes of 6, 9, and 12 thereafter. This was to further ensure less predictability of random allocation. Group allocations were concealed in envelopes that were opened by the study doctor once the participant had been enrolled in the study. The randomization envelopes were prepared by members of the vaccine group who were not involved in the study. The vaccines were given 6 months apart. Blood samples were obtained at 0, 1, 6, 7, 12, and 13 months before and 1 month after each vaccination.

**Vaccines**

PCV7 (Prevenar; Wyeth Vaccines; batch numbers: ND05370, NE31130, and NG12460) consisted of *Streptococcus pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F saccharides (4 μg of all serotypes except 6B and 6 μg of 6B) conjugated to a CRM197 carrier protein with aluminum phosphate as an adjuvant. 23vP (Pneumovax II; Aventis Pasteur; batch numbers: 20218, 25305, and 22995) consisted of *S. 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 (25 μg for each serotype). Both vaccines were given as 0.5-mL solutions intramuscularly using a 23G 25-mm needle.

After each vaccination, participants were asked to remain at the study center for 15 min in case of the event of an anaphylactic reaction. Any serious adverse event occurring during the study was recorded. The relationships of adverse events to the study vaccine were determined by the study investigators according to criteria of temporal relationship and biological plausibility.

**Laboratory Methods**

All blood samples collected were re-labeled with an independent label number before the samples were transferred to the laboratory. A randomized laboratory number was created for each study participant by the study statistician. This information was maintained on a password-controlled spreadsheet. This was to ensure that all study staff involved in processing and analyzing blood samples were blind to the vaccines received by the participant. Blood tubes were re-labeled by 2 members of the laboratory team, who ensured that the correct laboratory numbers was assigned to the correct sample.

Serum was separated within 24 h of sampling and was stored at ~80°C. Antigen-specific immunoglobulin G (IgG) concentrations were obtained with a validated bead-based multiplex assay that has been previously described [8]. Briefly,
pneumococcal polysaccharides were conjugated to carboxylated microspheres. Serum samples were then incubated with the conjugated microspheres, with cell wall polysaccharide and 22F polysaccharide. After the addition of anti-human IgG, beads were read on a Bio-plex using real-time Bio-plex software.

**Statistical Analysis**

The primary objective of this study was to assess the immunogenicity for 7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) 1 month after 23vP in each of the vaccine schedules, the null hypothesis being that there would be no difference with 0-, 1-, or 2-dose priming with PCV7. To detect a log difference of 0.6 in antibody between the groups with 80% power at a 2-sided level of significance of .002, a sample size of 105 individuals was required in each vaccine group. Allowing for a dropout rate of 10%, a total of 116 participants were required in each treatment group (348 in total).

The primary analysis was based on the principles of intention-to-treat. Comparisons between any of the 2 treatment groups were performed using analysis of covariance, adjusting for baseline antibody concentration and age at randomization. The P values for both primary and secondary objectives were adjusted for multiple comparisons using the False Discovery Rate Method [9]. Antibody concentrations were log transformed to obtain normality and were expressed as geometric mean concentrations (GMCs) with 95% confidence intervals (CIs). Statistical analysis was performed using Stata, version 9.1 (StataCorp).

**RESULTS**

A total of 348 participants were enrolled from October 2006 through June 2009 (Figure 1). The mean ages of participants in each group were similar, ranging from 58.8 to 59 years. The percentage of female subjects in each group varied from 52.2% to 53.6%. In total, 42 (12%) of the participants did not complete the study. During the 30-month study duration, there were 18 serious adverse events experienced by 17 participants; none of the adverse events were thought to be related to the study vaccines.

**Comparison of a Single Dose of 23vP versus PCV7 (Response to the Initial Vaccine Dose in Group 1 versus Groups 2 and 3)**

The GMCs of serotype specific IgG at 1 month after vaccination with PCV7 or 23vP were significantly higher for 4 of 7 serotypes (4, 9V, 18C, and 23F) in those individuals who were vaccinated with PCV7 (Table 1).

**The Response to 23vP after Priming with 1 or 2 Doses of PCV7**

Vaccination with a single dose of PCV7 prior to 23vP (PCV7-23vP) in group 2 induced serotype specific GMCs that did not differ significantly, at the 7-month time point, from those induced after an initial single dose of 23vP (group 1) at 1 month. At the 13-month time point, however, vaccination with 2 consecutive doses of PCV7 prior to vaccination with 23vP (PCV7-PCV7-23vP) in group 3 induced serotype-specific GMCs that were significantly higher for 3 of 7 serotypes (4, 9V, and 23F), compared with those induced by an initial single dose of 23vP at 1 month.

**The Response to PCV7 after Vaccination with 23vP**

At the 7-month time point, a dose of PCV7 preceded by a single dose of 23vP (23vP-PCV7) in group 1 produced lower serotype-specific IgG levels for all serotypes except 19F, compared with serotype-specific IgG levels in individuals who received an initial single dose of PCV7 (groups 1 and 3). This difference was significant for serotypes 4, 6B, 9V, 18C, and 23F. Furthermore, at 13 months, after 23vP followed by 2 doses of PCV7 (23vP-PCV7-PCV7), serotype specific IgG levels remained significantly lower than after a single initial dose of PCV7 for serotypes 4, 9V, 18C, and 23F (Table 1).

**DISCUSSION**

23vP provides limited protection against invasive pneumococcal disease in the elderly population, whereas PCV7 is highly effective in children but is limited to only 7 serotypes. Two previous studies have investigated schedules that use 2 doses of PCV7 to prime the immune system prior to vaccination with 23vP; these studies were conducted on human immunodeficiency virus–infected children and adults [10, 11]. This is, to our knowledge, the first study to consider such a schedule in the elderly population. Both the former studies reported incremental increases in GMC with each additional dose of vaccine but no clear booster response. In this study, combining PCV7 with the routinely used 23vP in older adults did not provide an immunological advantage using either a 1-dose or 2-dose priming schedule (PCV7-23vP, PCV7-PCV7-23vP). There are several explanations for the lack of booster responses to 23vP after 1 or 2 doses of PCV7. First, pre-existing immunity acquired as a result of nasopharyngeal colonization with pneumococci may have primed the polysaccharide specific B-cell response, such that PCV7 vaccination expands the B-cell population to a maximal threshold, preventing additional responses from being achieved by subsequent doses of 23vP or PCV7 [12]. A similar threshold seems to be reached in infants after 2 doses of PCV7, because a third priming dose does not further increase antibody concentrations significantly [13]. Second, high levels of antibody produced in response to the first dose of PCV7 may inhibit additional antibody production by a negative-feedback mechanism or neutralization of vaccine antigen [14–16]. Finally, and in contrast to the situation in infancy, it may be that PCV7 does not induce the immunological memory in adults that is
required to support a booster response, as suggested by Weller et al [17].

Earlier studies that compared the immunogenicity of PCV with that of 23vP in adults were inconclusive because of the different vaccine formulations used, the small study sizes, and the fact that only a limited number of serotypes were measured [18, 19]. In this study, we demonstrate that 1 dose of PCV7 is more immunogenic than 1 dose of 23vP for 4 of 7 serotypes contained in PCV7. This in keeping with more-recent studies that have shown that PCV7 is more immunogenic for 3–5 serotypes [6, 20, 21]. It is not clear whether the enhanced immunogenicity of PCV7 over 23vP for a limited number of serotypes provides a potential advantage in the elderly population for 2 reasons. First, in adults, both vaccines induce antibody concentrations for all serotypes that are higher than the correlate of protection in early childhood (0.35 µg/mL), but the level of antibody required to protect adults is unknown [22]. Furthermore, several studies have shown that, although adequate antibody concentrations may be obtained after pneumococcal vaccination in the elderly population, the functional activity of those antibodies is lower in this population than in younger adults. Therefore, measuring antibody function may

Figure 1. Consolidated Standards of Reporting Trials flow chart of participants through the study.
Table 1. Serotype-specific Immunoglobulin G (IgG) Geometric Mean Concentrations Prior to and 1 Month after Study Vaccination

<table>
<thead>
<tr>
<th>Vaccine schedule (no. of subjects)</th>
<th>4</th>
<th>6B</th>
<th>9V</th>
<th>14</th>
<th>18C</th>
<th>19F</th>
<th>23F</th>
</tr>
</thead>
<tbody>
<tr>
<td>23P-PCV7 (230)</td>
<td>0.16</td>
<td>1.41</td>
<td>0.42</td>
<td>2.75</td>
<td>0.37</td>
<td>5.69</td>
<td>1.49</td>
</tr>
<tr>
<td>(0, 1)</td>
<td>(.13–.18)</td>
<td>(1.13–1.76)</td>
<td>(.34–.52)</td>
<td>(2.05–3.69)</td>
<td>(.30–.45)</td>
<td>(4.39–7.38)</td>
<td>(1.17–1.90)</td>
</tr>
<tr>
<td>23vP (110)</td>
<td>0.13</td>
<td>0.70</td>
<td>0.40</td>
<td>2.58</td>
<td>0.31</td>
<td>2.72</td>
<td>0.93</td>
</tr>
<tr>
<td>(0, 1)</td>
<td>(.11–.17)</td>
<td>(.54–.93)</td>
<td>(.29–.55)</td>
<td>(1.75–3.90)</td>
<td>(.24–.41)</td>
<td>(1.99–3.70)</td>
<td>(.64–1.35)</td>
</tr>
<tr>
<td>23P-PCV7 (103)</td>
<td>0.51</td>
<td>0.51</td>
<td>1.01</td>
<td>1.67</td>
<td>1.84</td>
<td>2.47</td>
<td>5.09</td>
</tr>
<tr>
<td>(6, 7)</td>
<td>(.37–0.70)</td>
<td>(.38–.68)</td>
<td>(.64–1.59)</td>
<td>(1.11–2.49)</td>
<td>(1.38–2.46)</td>
<td>(1.84–3.32)</td>
<td>(3.15–8.22)</td>
</tr>
<tr>
<td>PCV7-23vP (110)</td>
<td>0.72</td>
<td>0.92</td>
<td>1.62</td>
<td>2.98</td>
<td>3.40</td>
<td>4.18</td>
<td>5.25</td>
</tr>
<tr>
<td>(6, 7)</td>
<td>(.53–.97)</td>
<td>(.66–1.27)</td>
<td>(1.05–2.46)</td>
<td>(2.00–4.44)</td>
<td>(2.41–4.48)</td>
<td>(3.01–5.82)</td>
<td>(3.59–7.67)</td>
</tr>
<tr>
<td>PCV7-PCV7 (99)</td>
<td>0.96</td>
<td>1.7</td>
<td>1.62</td>
<td>4.79</td>
<td>4.65</td>
<td>6.03</td>
<td>6.17</td>
</tr>
<tr>
<td>23P-PCV7-PCV7 (36)</td>
<td>0.49</td>
<td>0.73</td>
<td>1.73</td>
<td>3.06</td>
<td>2.22</td>
<td>2.79</td>
<td>6.32</td>
</tr>
<tr>
<td>(97)</td>
<td>(.36–.65)</td>
<td>(.56–.97)</td>
<td>(1.18–2.56)</td>
<td>(2.09–4.47)</td>
<td>(1.67–2.96)</td>
<td>(2.07–3.75)</td>
<td>(1.67–2.96)</td>
</tr>
<tr>
<td>PCV7-PCV7-PCV7 (97)</td>
<td>1.24</td>
<td>1.48</td>
<td>3.03</td>
<td>4.28</td>
<td>4.65</td>
<td>6.07</td>
<td>7.77</td>
</tr>
</tbody>
</table>

**NOTE.** Vaccines were administered 6 months apart, and blood was sampled from each participant at 6 time points during the study prior to and 1 month after each vaccination (at 0, 1, 6, 7, 12, and 13 months). Comparisons were made using analysis of covariance, and P values were adjusted using the false-discovery rate method. CI, confidence interval; PCV7, 7-valent pneumococcal conjugate vaccine; post, 1 month after the last vaccination listed; pre, just prior to last vaccination listed; 23vP, 23-valent pneumococcal plain polysaccharide vaccine.

- Six participants enrolled in this group did not have a blood test after the first vaccination because of prior history of pneumococcal vaccination.
- Two participants enrolled in this group did not take part in the first vaccination or blood draw, and therefore results were not available.
- One participant had an insufficient sample for testing.
- One participant withdrew before post-vaccination blood draw.
- Significantly higher compared with a single initial dose of 23vP in group 1: serotype 4, \( P < .001 \); serotype 9V, \( P < .001 \); serotype 18C, \( P = .009 \); and serotype 23, \( P = .002 \).
- Significantly higher compared with a single initial dose of 23vP in group 1: serotype 4, \( P = .007 \); serotype 18V, \( P = .01 \); serotype 23F, \( P = .01 \).
- Significantly lower compared with a single initial dose of PCV7 in groups 2 and 3 combined, serotype 4, \( P < .001 \); serotype 6B, \( P = .02 \); serotype 9V, \( P = .004 \); serotype 18C, \( P < .001 \); serotype 23F, \( P < .001 \).
- Significantly lower compared with a single initial dose of PCV7 in groups 2 and 3 combined, serotype 4, \( P = .001 \); serotype 9, \( P = .005 \); serotype 18, \( P < .001 \); serotype 23, \( P < .001 \).
better reflect any potential advantage of PCV7 [23–25]. The use of functional antibody measurements in determining vaccine effectiveness in adults has not yet been validated, and few data are available. The results of functional antibody testing in 2 previous studies reflected that of the antibody concentration; therefore, in this study, the addition of functional antibody data is unlikely to change the conclusion. Second, the use of PCV7 does not address the need to protect against the remaining 16 serotypes that are contained in 23vP, but the recent availability of PCVs containing 10 and 13 serotypes may overcome this limitation to some extent.

Administration of 23vP prior to PCV7 produced significantly attenuated antibody concentrations, compared with PCV7 alone, as has been demonstrated in previous studies [6, 20, 21]. This is the first study that has formally evaluated whether antibody hyporesponsiveness induced by 23vP can be recovered with a conjugate vaccine, but we were unable to overcome the poor responses in our cohort with PCV7. The lack of recovery over the 12-month period of the study may be related to the persistence of polysaccharide antigen, which may suppress additional immune responses and has been suggested as a potential mechanism in murine studies [26]. It is not clear how long 23vP induced hyporesponsiveness persists. Recent studies by Mushet et al [7, 27] have shown that, 5 years after receipt of 23vP, antibody concentrations elicited by subsequent doses of PCV7 and 23vP are restored close to the concentrations seen after primary vaccination. These data suggest that, if PCV were to be used in those already vaccinated with 23vP, the optimal time interval and dosage would need to be established.

Schedules combining 23vP and PCV7 do not enhance the antibody concentrations over those elicited by a single dose of either vaccine type. These data do not support the use of schedules combining 23vP and PCV7. Currently, such schedules are recommended in children at increased risk of pneumococcal infection, but there is little evidence to support this practice. Antibody concentrations induced by both vaccines wane rapidly in the first year after vaccination. Therefore, repeated doses of PCV7 may be necessary to sustain antibody concentrations. With the availability of PCVs covering 10 and 13 serotypes, the possibility of improved protection against pneumococcal disease in the elderly population must be explored in efficacy trials as soon as possible [28].

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Potential conflicts of interest. A.J.P has conducted clinical trials on behalf of Oxford University sponsored by manufacturers of pneumococcal vaccines, but does not accept any personal payments from vaccine manufacturers: honoraria, travel expenses, and grants for support of educational activities are paid to an educational/administrative fund held by the Department of Paediatrics, Oxford University. R.I. has received financial assistance from Wyeth Vaccines and GlaxoSmithKline to attend scientific meetings. All other authors: no conflicts.

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