Schedules for Pneumococcal Vaccination of Preterm Infants: An RCT

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BACKGROUND AND OBJECTIVE: Premature infants have a higher risk of invasive pneumococcal disease and are more likely to have lower vaccine responses compared with term infants. Increasingly, immunization schedules are including a reduced, 2-dose, pneumococcal conjugate vaccine priming schedule. Our goal was to assess the immunogenicity of 3 commonly used 13-valent pneumococcal conjugate vaccine (PCV13) priming schedules in premature infants and their response to a 12-month booster dose.

METHODS: Premature infants (<35 weeks’ gestation) were randomized to receive PCV13 at 2 and 4 months (reduced schedule); 2, 3, and 4 months (accelerated schedule); or 2, 4, and 6 months (extended schedule). All infants received a 12-month PCV13 booster. Serotype-specific pneumococcal immunoglobulin G (IgG) for PCV13 serotypes was measured by using enzyme-linked immunosorbent assay 1 month after the primary and booster vaccinations.

RESULTS: A total of 210 infants (median birth gestation, 29+6 weeks; range, 23+2–34+6 weeks) were included. After the primary vaccination, 75% (95% confidence interval [CI], 62–85), 88% (95% CI, 76–95), and 97% (95% CI, 87–99) of participants had protective antibody concentrations for at least one-half the PCV13 serotypes for the reduced, accelerated, and extended schedules, respectively. After the booster vaccination, participants receiving the extended schedule had significantly lower (P < .05) geometric mean concentrations compared with reduced (for 9 of 13 serotypes) and accelerated (for 4 of 13 serotypes) schedules, but nearly all participations, regardless of schedule or serotype, had seroprotective IgG concentrations.

CONCLUSIONS: A reduced priming schedule of PCV13 resulted in higher post-booster IgG concentrations but lower post-primary concentrations. The optimum vaccine schedule for preterm infants will therefore depend on when they are most at risk for invasive pneumococcal disease.

WHAT’S KNOWN ON THIS SUBJECT: Premature infants have a higher risk of invasive pneumococcal disease and are more likely to have lower vaccine responses compared with term infants. The optimal primary schedule to generate protective concentrations of pneumococcal antibodies in preterm infants is unknown.

WHAT THIS STUDY ADDS: This randomized controlled trial of a 13-valent pneumococcal conjugate vaccine schedule in preterm infants found that a reduced primary schedule resulted in higher post-booster, but lower post-primary, immunoglobulin G concentrations. The optimum schedule for preterm infants depends on when they are most at risk for invasive disease.
Premature infants are at increased risk of vaccine-preventable diseases, including a twofold risk of invasive pneumococcal disease compared with term infants.\textsuperscript{1–3} In most industrialized countries with established pneumococcal immunization programs, the 13-valent pneumococcal conjugate vaccine (PCV13) has superseded the 7-valent pneumococcal conjugate vaccine (PCV7) and has been shown to be highly immunogenic in term infants.\textsuperscript{4–6}

The immunogenicity of PCV13 in premature infants receiving a 2–3–4 and 12-month schedule was only recently reported and revealed lower immunoglobulin G (IgG) concentrations for 8 serotypes after both primary and booster doses compared with term infants.\textsuperscript{7} This lower immunogenicity is consistent with previous PCV7 studies\textsuperscript{8–10} and is concerning because premature infants are also less likely to benefit from the protective maternal antibodies transferred during late pregnancy.

In addition, national immunization programs are increasingly including reduced 2 dose priming schedules.\textsuperscript{11,12} These schedules are immunogenic in term infants and, with some vaccines, may even improve B-cell memory and booster responses.\textsuperscript{13–16} However, little is known about the immunogenicity of fewer primary doses in premature infants.

The goal of the present randomized controlled trial was to assess the immunogenicity of reduced, accelerated (intended to provide maximum early protection), and extended (doses administered over a longer period) PCV13 priming schedules in premature infants after completion of the primary series and after a 12-month booster.

\textbf{METHODS}

\textbf{Participants and Recruitment}

Premature infants were enrolled in a Phase IV, open-label, randomized controlled trial from 12 centers in the United Kingdom between May 2012 and May 2013. Potentially eligible infants were identified by the clinical teams, and parents were provided with information by the research teams. Infants were eligible for inclusion if they had a birth gestation <35\textsuperscript{th} weeks, had no contraindications for vaccination as defined by Department of Health guidelines,\textsuperscript{17} and were between 7 and 12 weeks of age. In addition, infants should not have received any other vaccinations (with the exceptions of BCG and hepatitis B). Information on the participants’ medical, medication, and vaccination history was collected from the medical records by using a standardized case-report form.

Written informed consent was obtained from parents before enrollment. The study was approved by the East of England–Essex research ethics committee (REC reference 07/H0301.11).

\textbf{Vaccination}

Infants were randomly assigned (1:1:1) to receive PCV13 (Prevenar 13; Pfizer, New York, NY) at 2 and 4 months of age (reduced schedule, group 1); at 2, 3, and 4 months of age (accelerated schedule, group 2); or at 2, 4, and 6 months of age (extended schedule, group 3) (Supplemental Table 4). A booster dose of PCV13 was administered to all infants at 12 months of age. In addition, all participants received a combined diphtheria, tetanus, acellular pertussis, \textit{Haemophilus influenzae} type b, and inactivated polio vaccine (Pediacel; Sanofi Pasteur MSD, Lyon, France) at 2, 3, and 4 months of age; a meningococcal \textit{C}–\textit{CRM}197 vaccine (Menjugate; Novartis Vaccines, Siena, Italy) at 3 and 4 months of age; and a combined measles, mumps, and rubella vaccine (Priorix; GlaxoSmithKline Biologicals, Rixensart, Belgium) and combined \textit{H influenzae} type b and \textit{Neisseria meningitidis} serogroup C tetanus toxoid (Hib-MenC-TT) conjugate vaccine (Menitorix; GlaxoSmithKline Biologicals) at 12 months of age. Participants were vaccinated in the hospital if still receiving inpatient care. All vaccines were administered intramuscularly.

Computerized block randomization was stratified according to center and gestation (<30 or ≥30 weeks’ gestation), and each center was allocated blocks of sequential numbers (block size 18). After consent, the subject was allocated the next available study number for that center and gestational age cohort, and the appropriate sealed envelope containing the group allocation was opened. The study was not blinded to parents or clinical personnel.

\textbf{Blood Sampling and Serologic Methods}

Up to 3 mL of whole blood was obtained from each participant before the first vaccination (baseline), 1 month after the primary vaccination (at age 5 months for groups 1 and 2 participants and at age 7 months for group 3 participants), and before and 1 month after booster vaccination (12 and 13 months, respectively) (Supplemental Table 4). Serologic analysis was performed at the World Health Organization reference laboratory for pneumococcal serology, Institute of Child Health, London. After extraction from whole blood, sera were stored at −70°C before assaying for pneumococcal serotype-specific IgG concentrations for the PCV13 pneumococcal serotypes by using an enzyme-linked immunosorbent assay as previously described.\textsuperscript{18} The lower limit of assay quantification was 0.15 μg/mL, and IgG concentrations ≥0.35 μg/mL were considered protective.\textsuperscript{19}
**Safety Analysis**

All participants were observed for immediate adverse reactions. Solicited systemic and local adverse reactions were recorded by the infant’s main caregiver for 7 days after each vaccination. All adverse events (AEs), including serious AEs, were recorded for 28 days after each vaccination by using an AE diary. Parents had access to a 24-hour telephone contact number for AE reporting.

**Statistical Analysis**

The primary objectives were to assess IgG geometric mean concentrations (GMCs) and the proportion of infants with protective serotype-specific antibody concentrations for PCV13 serotypes at 1 month after completion of the primary vaccination course, according to the 3 schedules. The main secondary objectives were to assess differences in serotype-specific IgG GMC and seroprotection rates between schedules before and after booster vaccination at 12 months of age, and to quantify the percentage of children experiencing fever, local reactions, and nonfebrile systemic reactions within 7 days after each vaccine dose.

Pretrial sample size calculations estimated a minimum of 60 infants in each group to detect at least a twofold difference between groups after primary immunization, with 80% power and 5% significance. Based on published data, the SD of IgG responses was estimated to be 0.6 log10 units. To allow for withdrawal of subjects over the course of the study and the challenges of obtaining blood samples from very premature infants, we aimed to recruit 210 infants.

Data were analyzed by using a modified intention-to-treat analysis including all infants who received a dose of PCV13 and from whom at least 1 postvaccination blood sample was obtained. GMCs and 95% confidence intervals (CIs) were calculated for each sampling time point, along with the proportion of infants achieving protective antibody concentrations and binominal CI. Results below the lower limit of quantification were taken to be one-half this level for computational purposes.

Statistical comparison of antibody concentrations and the proportion of participants with protective concentrations or AEs between the 3 trial arms were performed by using Student’s t test and the χ² test or Fisher’s exact test, as appropriate. Statistical significance was defined as P < .05. To facilitate comparisons, schedules were analyzed based on the proportions achieving adequate protection for at least one-half of the serotypes. The number of serotypes with protective concentrations per participant was compared by using the nonparametric Kruskal-Wallis one-way analysis of variance test.

Logistic regression was used to examine the effect of gestation, the receipt of antenatal or postnatal steroids, blood transfusion, BCG vaccine, early postvaccination paracetamol, and the presence of chronic lung disease (defined as requiring oxygen or respiratory support at 28 days of age) on seroprotection. Analysis was adjusted for gestation. For post-primary vaccination results, multivariable linear regression using log-transformed values was performed (adjusting for group and gestation). Logistic regression was not performed on baseline IgG concentrations due to the large number of results below the lower limit of quantification.

All data were analyzed by using Stata version 13 (Stata Corp, College Station, TX).

**RESULTS**

A total of 210 infants were recruited; 199 participants (94.7%) completed the primary phase (primary end point), and 194 (92.4%) completed the entire study (Fig 1). Two participants died of causes unrelated to the trial. The majority of infants who did not meet the inclusion criteria were outside the study age range or were too unstable for vaccination. A second group of infants was excluded for logistical reasons (many were transferred to their local neonatal unit before their first vaccination).

The characteristics of randomized infants were similar between groups (Table 1), with a median birth gestation of 29+6 weeks (range, 23+2–34+6 weeks) and a median birth weight of 1388 g (range, 450–3390 g). A total of 112 vaccinations were administered to hospitalized participants.

**Primary Vaccination**

At baseline, participants had low antibody concentrations for all pneumococcal serotypes (Table 2, Supplemental Table 5). The highest IgG GMCs (for all participants) were seen for serotypes 14 (0.26 μg/mL) and 19A (0.19 μg/mL).

After the primary vaccination course, substantial increases in antibody concentrations were seen for all serotypes and all groups. There was considerable variation between serotypes, with IgG GMCs ranging from 0.16 μg/mL for serotype 6B (reduced schedule) to 8.49 μg/mL for serotype 14 (extended schedule) (Fig 2, Supplemental Table 6).

The primary schedule had a significant impact on vaccine immunogenicity. Lack of seroprotection for more than one-half of the PCV13 serotypes was seen in 25%, 12%, and 3% of participants receiving the reduced, accelerated, and extended schedules, respectively (P < .001) (Supplemental Fig 4, Supplemental Table 7).
Participants receiving the extended schedule had higher IgG GMCs compared with the reduced schedule for 11 serotypes and accelerated schedule for 7 serotypes. The accelerated schedule was superior to the reduced schedule for 4 serotypes (Fig 2, Table 2, Supplemental Table 6).

**Booster Vaccination**

At 12 months of age, waning of pneumococcal antibody
concentrations was evident with low rates of seroprotection against individual serotypes (Table 3, Supplemental Table 8). Antibody concentrations remained significantly higher in those who had received the extended schedule compared with those receiving the reduced (for 10 serotypes) or accelerated (for 11 serotypes) schedules; the accelerated schedule was superior to the reduced schedule for 1 serotype only.

After booster vaccination, a high proportion of infants achieved protective concentrations (Table 3). Similar to findings from previous time points, significant variation in antibody concentrations between serotypes and groups was apparent (Fig 3). In contrast to post-primary vaccination responses, participants receiving the extended schedule had lower GMCs compared with those receiving the reduced (for 9 serotypes) and accelerated (for 4 serotypes) schedules. The accelerated schedule was inferior to the reduced schedule for 1 serotype (19A) (Supplemental Table 9). Infants who received the extended schedule had lower fold increases in concentrations after booster vaccination than the other groups (Supplemental Fig 5).

Data are presented as median (range) or n (%). CLD, chronic lung disease.

### TABLE 1 Participant Characteristics According to Group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Reduced Dose (Group 1), n = 68</th>
<th>Accelerated (Group 2), n = 67</th>
<th>Extended (Group 3), n = 71</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation, wk</td>
<td>29.6 (24.9–34.9)</td>
<td>30 (23.6–34.9)</td>
<td>30 (23.3–34.9)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>1410 (576–2600)</td>
<td>1550 (510–3390)</td>
<td>1390 (450–2680)</td>
</tr>
<tr>
<td>Weight at first vaccination, g</td>
<td>2442 (845–4660)</td>
<td>2350 (1260–5070)</td>
<td>2497 (920–4560)</td>
</tr>
<tr>
<td>Male sex</td>
<td>37 (54)</td>
<td>32 (48)</td>
<td>38 (54)</td>
</tr>
<tr>
<td>Ethnicity, white</td>
<td>57 (84)</td>
<td>54 (81)</td>
<td>60 (85)</td>
</tr>
<tr>
<td>CLD</td>
<td>23 (34)</td>
<td>22 (33)</td>
<td>27 (38)</td>
</tr>
<tr>
<td>Antenatal steroids</td>
<td>59 (87)</td>
<td>56 (84)</td>
<td>62 (87)</td>
</tr>
<tr>
<td>Postnatal steroids</td>
<td>4 (6)</td>
<td>4 (6)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>28 (41)</td>
<td>30 (45)</td>
<td>29 (41)</td>
</tr>
<tr>
<td>BCG vaccine</td>
<td>5 (7)</td>
<td>5 (7)</td>
<td>7 (10)</td>
</tr>
<tr>
<td>Age, d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At visit 1</td>
<td>61 (49–86)</td>
<td>61 (49–83)</td>
<td>61 (46–88)</td>
</tr>
<tr>
<td>At visit 2</td>
<td>93 (78–136)</td>
<td>93 (82–119)</td>
<td>95 (79–132)</td>
</tr>
<tr>
<td>At visit 3</td>
<td>126 (111–178)</td>
<td>126 (114–160)</td>
<td>126 (106–160)</td>
</tr>
<tr>
<td>At visit 4</td>
<td>158 (132–198)</td>
<td>158 (135–187)</td>
<td>—</td>
</tr>
<tr>
<td>At visit 5</td>
<td>—</td>
<td>—</td>
<td>181 (156–258)</td>
</tr>
<tr>
<td>At visit 6</td>
<td>—</td>
<td>—</td>
<td>209 (177–298)</td>
</tr>
<tr>
<td>At visit 7</td>
<td>368 (353–410)</td>
<td>367 (351–404)</td>
<td>368 (351–429)</td>
</tr>
<tr>
<td>At visit 8</td>
<td>400 (367–443)</td>
<td>400 (357–492)</td>
<td>397 (375–608)</td>
</tr>
</tbody>
</table>

Data are given as proportion (95% CI). Comparison of (P < .05):
- accelerated and extended schedules,
- reduced and extended schedules, and
- reduced and accelerated schedules

### TABLE 2 Proportion of Infants With Protective Antibody Concentrations (IgG ≥ 0.35 μg/mL) for the 13 PCV13 Serotypes at Baseline and 1 Month After Final Primary Vaccination

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Baseline</th>
<th>Post-Primary Immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All, N = 197</td>
<td>Reduced Dose (Group 1), n = 66</td>
</tr>
<tr>
<td>1</td>
<td>0.03 (0.01–0.07)</td>
<td>0.85 (0.74–0.92)</td>
</tr>
<tr>
<td>3</td>
<td>0.01 (0.00–0.03)</td>
<td>0.61 (0.48–0.73)</td>
</tr>
<tr>
<td>4</td>
<td>0.02 (0.01–0.05)</td>
<td>0.92 (0.83–0.97)</td>
</tr>
<tr>
<td>5</td>
<td>0.02 (0.01–0.05)</td>
<td>0.36 (0.25–0.49)</td>
</tr>
<tr>
<td>6A</td>
<td>0.13 (0.08–0.19)</td>
<td>0.58 (0.45–0.70)</td>
</tr>
<tr>
<td>6B</td>
<td>0.07 (0.04–0.11)</td>
<td>0.20 (0.11–0.31)</td>
</tr>
<tr>
<td>7F</td>
<td>0.05 (0.02–0.09)</td>
<td>0.91 (0.81–0.97)</td>
</tr>
<tr>
<td>9V</td>
<td>0.06 (0.03–0.10)</td>
<td>0.59 (0.46–0.71)</td>
</tr>
<tr>
<td>14</td>
<td>0.38 (0.31–0.45)</td>
<td>0.94 (0.85–0.98)</td>
</tr>
<tr>
<td>18C</td>
<td>0.05 (0.02–0.08)</td>
<td>0.88 (0.78–0.95)</td>
</tr>
<tr>
<td>19A</td>
<td>0.24 (0.18–0.30)</td>
<td>0.83 (0.72–0.91)</td>
</tr>
<tr>
<td>19F</td>
<td>0.14 (0.09–0.19)</td>
<td>0.97 (0.89–1.00)</td>
</tr>
<tr>
<td>23F</td>
<td>0.06 (0.03–0.10)</td>
<td>0.47 (0.34–0.60)</td>
</tr>
</tbody>
</table>

Data are given as proportion (95% CI). Comparison of (P < .05):
- accelerated and extended schedules,
- reduced and extended schedules, and
- reduced and accelerated schedules

* P < .001.
Predictors of Antibody Concentrations

Increased odds of seroprotection at 2 months of age were seen with each week of increased gestation for 4 serotypes: 6A (odds ratio [OR], 1.34 [95% CI, 1.12–1.60]; \( P = .001 \)), 14 (OR, 1.25 [95% CI, 1.12–1.45]; \( P < .001 \)), and 19F (OR, 1.29 [95% CI, 1.08–1.52]; \( P = .003 \)). Later gestation was associated with an increase in post-primary vaccination IgG concentrations for 3 serotypes: 1 (6% increase per week [95% CI, 0.9–12]; \( P = .021 \)), 3 (8% increase per week [95% CI, 4–14]; \( P < .001 \)), and 7F (8% increase per week [95% CI, 3–13]; \( P = .002 \)).

Receipt of antenatal steroids was associated with decreased odds of seroprotection at 2 months for 4 serotypes: 5 (OR, 0.09 [95% CI, 0.01–0.83]; \( P = .033 \)), 6A (OR, 0.26 [95% CI, 0.10–0.69]; \( P = .006 \)), 19A (OR, 0.19 [95% CI, 0.08–0.45]; \( P < .001 \)), and 23F (OR, 0.23 [95% CI, 0.06–0.80]; \( P = .021 \)). In addition, post-primary vaccination serotype-specific IgG GMCs for serotypes 1, 4, and 9V were reduced in infants who had been exposed to antenatal steroids. At no time points were antenatal steroids associated with higher antibody concentrations.

Pre- or post-primary protective concentrations were not associated with any other factors in the regression analysis. Too few infants were exposed to postnatal steroids to analyze any effect. Serotype-specific antibody concentrations after the 12-month PCV13 booster were affected by priming schedule and preexisting antibody levels only.

Safety and AEs

There were no significant differences in the frequency or severity of

### TABLE 3 Proportion of Infants With Protective Antibody Concentrations (IgG ≥0.35 μg/mL) Before Booster Vaccination (12 Months) and 1 Month After Booster Vaccination

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Pre-Booster Vaccination</th>
<th>Post-Booster Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reduced Dose (Group 1), ( n = 64 )</td>
<td>Accelerated (Group 2), ( n = 57 )</td>
</tr>
<tr>
<td>1</td>
<td>0.23 (0.14–0.36)</td>
<td>0.19 (0.10–0.32)</td>
</tr>
<tr>
<td>3</td>
<td>0.18 (0.09–0.30)</td>
<td>0.22 (0.12–0.35)</td>
</tr>
<tr>
<td>4</td>
<td>0.11 (0.05–0.21)</td>
<td>0.11 (0.04–0.22)</td>
</tr>
<tr>
<td>5</td>
<td>0.20 (0.11–0.32)</td>
<td>0.14 (0.06–0.26)</td>
</tr>
<tr>
<td>6A</td>
<td>0.33 (0.27–0.52)</td>
<td>0.38 (0.25–0.51)</td>
</tr>
<tr>
<td>6B</td>
<td>0.19 (0.10–0.30)</td>
<td>0.16 (0.08–0.29)</td>
</tr>
<tr>
<td>7F</td>
<td>0.64 (0.51–0.76)</td>
<td>0.68 (0.54–0.80)</td>
</tr>
<tr>
<td>9V</td>
<td>0.06 (0.02–0.15)</td>
<td>0.08 (0.03–0.19)</td>
</tr>
<tr>
<td>14</td>
<td>0.86 (0.75–0.93)</td>
<td>0.95 (0.85–0.99)</td>
</tr>
<tr>
<td>18C</td>
<td>0.06 (0.02–0.15)</td>
<td>0.09 (0.03–0.20)</td>
</tr>
<tr>
<td>19A</td>
<td>0.39 (0.27–0.53)</td>
<td>0.57 (0.43–0.70)</td>
</tr>
<tr>
<td>19F</td>
<td>0.63 (0.50–0.75)</td>
<td>0.49 (0.35–0.63)</td>
</tr>
<tr>
<td>23F</td>
<td>0.15 (0.07–0.26)</td>
<td>0.11 (0.04–0.22)</td>
</tr>
</tbody>
</table>

Data are given as proportion (95% CI).
Comparison of (\( P < 0.05 \));
\(^a\) accelerated and extended schedules,
\(^b\) reduced and extended schedules
\(^*\) \( P < .001 \).
To the best of our knowledge, this study is the first to compare different PCV13 schedules in premature infants, and it shows the need for early and effective immunization strategies for this vulnerable group, given their very low preimmunization antibody concentrations. Our results indicate that most preterm infants can achieve seroprotective antibody concentrations for the serotypes in PCV13 regardless of the primary schedule administered, especially after the 12-month booster, but the magnitude of their immunologic response is dependent on the primary schedule they receive.

Serotype-specific responses varied, with lower IgG GMCs achieved for serotypes 3, 5, and 6B after the primary course and for serotypes 3, 9V, and 18C after the booster dose; these findings are consistent with those observed in term infants. However, compared with previous term (PCV13) and preterm (PCV7) studies, antibody concentrations after primary and booster vaccination are lower overall, resulting in lower seroprotection after primary vaccination. Similarly, compared with the recent PCV13 preterm study, lower IgG GMCs and seroprotection rates were seen for all serotypes. These differences may be due to the different laboratory testing methods used for serotype-specific antibody concentrations, but potential biological explanations include interactions with concurrently administered vaccines, the younger gestation of the study cohort, or our broad inclusion criteria encompassing infants with complex medical problems (representative of the preterm population). In addition, in their recent study, Martinón-Torres et al did not report baseline IgG concentrations, which may differ between countries and affect postvaccination concentrations.

When comparing schedules within the study cohort, the most striking finding was the contrasting immunogenicity of the 3 schedules at different time points, with the reduced dose schedule generating inferior antibody concentrations after the primary course but superior antibody concentrations after the booster dose. The higher post-primary IgG GMCs after 3 doses (compared with 2 doses) is consistent with 2 meta-analyses of primary schedules in term infants. Of the 3-dose schedules, higher antibody concentrations were seen in premature infants receiving the extended schedule. This finding was not observed in the meta-analyses of term infant responses, but an older age at final vaccination may be more important in premature infants because it will allow further maturation of their immune system. However, this scenario needs to be set against the optimal age at which protection is required in this population. Several studies have indicated an increased susceptibility of invasive pneumococcal disease in infants born prematurely compared with term infants; this risk seems maximal in the first 6 months of life.

The differences in response to the booster dose were unexpected because type of priming schedule...
has not been consistently shown to affect the generation of immunologic memory and pneumococcal conjugate vaccine booster responses in term infants.\(^{23,27}\) The improved post-booster immunogenicity of fewer priming doses is well described for meningococcal C conjugate vaccines and is believed to be due to lower total antigen exposure favoring differentiation of B lymphoblasts into memory B cells instead of antibody-generating plasma cells.\(^{14,15}\) In pneumococcal conjugate vaccines, a study of Fijian infants receiving 1 PCV7 priming dose followed by the 23-valent pneumococcal polysaccharide vaccine at 12 months had higher IgG GMCs for serotypes 4, 9V, and 19F compared with those who had been primed with 2 or 3 PCV7 doses.\(^{13}\) Similarly, infants receiving a lower antigen-containing investigational tetravalent pneumococcal conjugate vaccine for priming had higher booster responses than those who had received the higher antigen-containing preparation.\(^{28}\) However, it should be noted that a statistically significant difference between the reduced and accelerated schedule groups was observed for only 1 serotype.

Despite seroprotective concentrations, infants who had received the extended schedule had lower fold increases in antibody concentrations after booster vaccination than those receiving either the reduced dose or accelerated schedule, suggesting that the higher pre-booster antibody concentrations at 12 months may have interfered with booster responses. This effect has been observed after booster doses for other vaccines, and several hypotheses have been proposed. These hypotheses include the formation of immune complexes consisting of preexisting antibody and vaccine antigen resulting in less available vaccine antigen and B-cell receptor–mediated negative feedback mechanisms, analogous to those described for high maternal antibody concentrations impairing primary vaccine responses.\(^{29-33}\)

Within this cohort of premature infants, increasing birth gestation was associated with increased immunogenicity. This outcome has previously been described for other vaccines and reflects deficiencies in both the innate and adaptive immune systems in these more premature infants.\(^{34-39}\) The present study had some potential limitations. The varying ages of infants at blood sampling between the groups must be considered when comparing primary schedules; the antibody concentrations at 7 months for infants in groups 1 and 2 are not known. It is possible that infants in those groups may have had an increase in their antibody concentrations between their 5-month sample and 7 months of age due to natural exposure.\(^{40}\) However, a study comparing schedules in term infants, which sampled some infants at both 5 and 8 months, found no increase in antibodies between these ages.\(^{27}\) We also did not measure antibody concentrations beyond 13 months of age.

Because the objectives of the present study were to look at schedule differences within the premature population, we did not include a term comparator group. However, lower antibody concentrations were seen in our study cohort compared with a recent cohort of term infants in the United Kingdom who received a reduced dose schedule, which was analyzed in the same laboratory.\(^{22}\) In addition, we did not include any assessment of functional activity of the antibodies detected. Opsonophagocytic antibody titers may have allowed us to assess the potential clinical impact of schedule differences in more detail and should be considered in future studies. A previous meta-analysis of primary pneumococcal conjugate vaccine schedules in term infants has shown a good relationship between enzyme-linked immunosorbent assay–measured IgG concentrations and opsonophagocytic antibody titers. However, an analysis of serotype-specific opsonophagocytic activity did not find a consistent protective opsonophagocytic activity titer across all vaccine serotypes.\(^{24,41}\)

**CONCLUSIONS**

PCV13 was well tolerated in premature infants. Different priming schedules resulted in higher IgG concentrations at different times during the first 13 months of life. We believe that such data will be beneficial to those planning or providing pneumococcal vaccines to preterm infants and will enable them to consider this finding in the context of their own immunization programs and epidemiologic situations.

**ACKNOWLEDGMENTS**

The authors thank all children who participated in the study as well as their parents/guardians; Dr Nicola Pritchard and the study staff at all the research centers; and Pauline Kaye, Deborah Cohen, Teresa Gibbs, and all other members of the team at Public Health England and NIHR CRN for their invaluable help and support. The authors also thank Pfizer for financially supporting this investigator-led study.

Members of the PUNS study group were as follows: Gill Ager, Neonatal Unit, Queen Alexandra Hospital, Portsmouth, United Kingdom; Matthew D. Snape, Oxford Vaccine Group, University of Oxford, and the NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom; Karen Few, Neonatal Unit, Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich,
This work was presented in part at the annual meeting of the European Society for Paediatric Infectious Diseases, May 8-10, 2014; Dublin, Ireland, and at the annual meeting of the European Society for Paediatric Infectious Diseases, May 12-16, 2015; Leipzig, Germany.

Dr Kent coordinated the study, performed statistical analysis, and drafted the initial manuscript; Dr Ladhani assisted with the design of the study, coordination of the study, and critical review of the manuscript; Dr Andrews approved the data collection tools, performed the statistical analysis, and critically reviewed the manuscript; Drs Scorrer, Pollard, Clarke, Hughes, Heal, Merson, Chang, Satodia, Collinson, and Faust were members of the trial steering committee, recruited participants, were responsible for data collection and study procedures at their sites, and critically reviewed the manuscript; Dr Goldblatt supervised the analysis of all laboratory samples and critically reviewed the manuscript; and Prof Miller and Prof Heath were responsible for the concept and design of the study, the overall supervision of all aspects of the clinical trial, and critical review of the manuscript; and all authors approved the final manuscript as submitted.

This trial has been registered with the European Union Clinical Trials Register (EudraCT number 2007-007535-23).

DOI: 10.1542/peds.2015-3945

Accepted for publication May 27, 2016

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

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FINANCIAL DISCLOSURE: Dr Ladhani and Prof Heath have conducted studies on behalf of St George’s, University of London, funded by vaccine manufacturers but do not receive any personal payments or travel support. Prof Pollard has previously conducted clinical trials on behalf of Oxford University, funded by vaccine manufacturers, but did not receive any personal payments from them. Prof Pollard chairs the UK Department of Health’s Joint Committee on Vaccination and Immunization; the views expressed in this manuscript do not necessarily reflect the views of the Joint Committee on Vaccination and Immunization or the UK Department of Health. Dr Faust acts as chief or principal investigator for clinical trials and studies conducted on behalf of the University Hospital Southampton NHS Foundation Trust and the University of Southampton, sponsored by vaccine manufacturers, universities, or NHS trusts but receives no personal payments from them. Dr Faust has participated in advisory boards for vaccine manufacturers but receives no personal payments for this work; all grants and honoraria are paid into accounts at the NHS trust or university. Dr Goldblatt’s UCL Institute of Child Health receives funding for contract research from GlaxoSmithKline; he contributes to occasional GlaxoSmithKline advisory boards; and he is supported by the National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London. The other authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: This work was supported by Pfizer Ltd as an investigator-led study. The funder had no input into the conduct of the trial, analysis of data, interpretation of results, or the preparation of this manuscript.

POTENTIAL CONFLICT OF INTEREST: Dr Ladhani and Prof Heath have conducted studies on behalf of St George’s, University of London, funded by vaccine manufacturers but do not receive any personal payments or travel support. Prof Pollard has previously conducted clinical trials on behalf of Oxford University, funded by vaccine manufacturers, but did not receive any personal payments from them. Prof Pollard chairs the UK Department of Health’s Joint Committee on Vaccination and Immunization; the views expressed in this manuscript do not necessarily reflect the views of the Joint Committee on Vaccination and Immunization or the UK Department of Health. Dr Faust acts as chief or principal investigator for clinical trials and studies conducted on behalf of the University Hospital Southampton NHS Foundation Trust and the University of Southampton, sponsored by vaccine manufacturers, universities, or NHS trusts but receives no personal payments from them. Dr Faust has participated in advisory boards for vaccine manufacturers. Dr Goldblatt contributes to occasional GlaxoSmithKline advisory boards; he is supported by the National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London. The other authors have indicated they have no potential conflicts of interest to disclose.

COMPANION PAPER: A companion to this article can be found online at www.pediatrics.org/cgi/doi/10.1542/peds.2016-0975.

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Schedules for Pneumococcal Vaccination of Preterm Infants: An RCT
Alison Kent, Shamez N. Ladhani, Nick J. Andrews, Tim Scorrer, Andrew J. Pollard, Paul Clarke, Stephen M. Hughes, Carrie Heal, Esse Menson, John Chang, Prakash Satodia, Andrew C. Collinson, Saul N. Faust, David Goldblatt, Elizabeth Miller, Paul T. Heath and on behalf of the PUNS Study Group
Pediatrics 2016;138; originally published online August 8, 2016;
DOI: 10.1542/peds.2015-3945

The online version of this article, along with updated information and services, is located on the World Wide Web at:
/content/138/3/e20153945.full.html