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Dr S Ladhani and Prof P T 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 A J 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 A J Pollard chairs the UK Department of Health's (DH) Joint Committee on Vaccination and Immunization (JCVI); the views expressed in this manuscript do not necessarily reflect the views of JCVI or DH.
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Dr S Ladhani and Prof P T 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.
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Abbreviations:

PCV7	7 valent pneumococcal conjugate vaccine
PCV13	13 valent pneumococcal conjugate vaccine
IgG	Immunoglobulin G
GMC	Geometric mean concentrations
IPD	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 to term infants. The optimal primary schedule to generate protective concentrations of pneumococcal antibodies in preterm infants is unknown.

What this study adds:
This 13-valent pneumococcal conjugate vaccine schedule RCT in preterm infants demonstrated that fewer priming doses resulted in higher post-booster, but lower post-primary IgG concentrations. The optimum schedule for preterm infants depends when they are most at risk of invasive disease.

Contributors' Statement Page:

Dr A Kent coordinated the study, performed statistical analysis and drafted the initial manuscript.
Dr S Ladhani assisted with the design of the study, coordination of the study, critically reviewed the manuscript and approved the final manuscript as submitted.

Dr N Andrews approved the data collection tools, performed the statistically analysis, critically reviewed the manuscript and approved the final manuscript as submitted.

Dr T Scorrer, Prof A Pollard, Dr P Clarke, Dr S Hughes, Dr C Heal, Dr E Menson, Dr J Chang, Dr P Satodia, Dr A C Collinson, Dr N Pritchard and Dr S Faust were members of the trial steering committee, recruited participants and were responsible for data collection and study procedures at their sites. They critically reviewed the manuscript and approved the final manuscript as submitted.

Prof D Goldblatt supervised the analysis of all laboratory samples, critically reviewed the manuscript and approved the final manuscript as submitted.

Prof E Miller and Prof P T Heath were responsible for the concept and design of the study and the overall supervision of all aspects of the clinical trial. They critically reviewed the manuscript and approved the final manuscript as submitted.

Abstract

Background

Premature infants have a higher risk of invasive pneumococcal disease and are more likely to have lower vaccine responses compared to term infants. Increasingly, immunization schedules are including a reduced, 2-dose, pneumococcal conjugate vaccine (PCV) priming schedule.

We aimed to assess the immunogenicity of 3 commonly used 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 were measured by ELISA 1 month after primary and booster vaccinations.

Results

A total of 210 infants (median birth gestation 29^{+6} weeks, range 23^{+2}-34^{+6}) were included. Following primary vaccination, 75% (95% CI 62-85), 88% (95% CI 76-95) and 97% (95% CI 87-99) of participants had protective antibody concentrations for at least half the PCV13 serotypes for the reduced, accelerated and extended schedules respectively. Following booster vaccination, participants receiving the extended schedule had significantly lower ($p<0.05$) geometric mean concentrations compared with reduced (for 9/13 serotypes) and accelerated schedules (for 4/13 serotypes).

Conclusions

Fewer priming doses of PCV13 resulted in lower post-primary concentrations but higher post-booster IgG concentrations than an extended schedule. The optimum vaccine schedule for preterm infants will therefore depend on when they are most at risk of invasive pneumococcal disease.

Introduction

Premature infants are at increased risk of vaccine preventable diseases, including a two-fold risk of invasive pneumococcal disease (IPD) compared to term infants.[1–3]

In most industrialised countries with established pneumococcal immunization programmes, the 13-valent pneumococcal conjugate vaccine (PCV13) has superseded the 7-valent PCV and has been shown to be highly immunogenic in term infants.[4–6]

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

Additionally, national immunization programmes are increasingly including reduced (2) dose priming schedules.[11,12] These schedules are immunogenic in term infants and, with some vaccines, may even improve B cell memory and booster responses.[13–16] However, little is known about the immunogenicity of fewer primary doses in premature infants.

This randomized, controlled trial aimed 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.

Patients and Methods

Participants and recruitment

Premature infants were enrolled in a phase IV open-label randomized controlled trial from 12 UK centres 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 less than 35^{+0} weeks, had no contra-indications for vaccination as defined by Department of Health guidelines[17] and were between 7 and 12 weeks of age. Additionally, infants should not have received any other vaccinations (with the exceptions of BCG and hepatitis B). Information on the participants' past medical, medication and vaccination history was collected from the medical records using a standardised case report form.

Written informed consent was obtained from parents prior to enrolment. The study was approved by the East of England – Essex research ethics committee (REC reference 07/HO301.11) and registered on the EudraCT clinical trial database (2007-007535-23).

Vaccination

Infants were randomly assigned (1:1:1) to receive PCV13 (Prevenar13; Pfizer, New York) 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)(supplementary table 1). A booster dose of PCV13 was administered to all infants at 12 months of age. Additionally, all participants received a combined diphtheria, tetanus, acellular pertussis, *Haemophilus influenzae* type b and inactivated polio vaccine (Pediacel; Sanofi Pasteur MSD, Lyon, France) at 2, 3 and 4 months old, meningococcal C-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 Hib-MenC-TT conjugate vaccine (Menitorix, GlaxoSmithKline Biologicals, Rixensart, Belgium) at 12 months of age (supplementary table 1). Participants were vaccinated in hospital if still receiving inpatient care. All vaccines were administered intramuscularly.

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

Blood sampling and serological methods

Up to 3 mL of whole blood was obtained from each participant prior to the first vaccination (baseline), 1 month following primary vaccination (at age 5 months for Groups 1 and 2 participants and at age 7 months for Group 3 participants), prior to and 1 month after booster vaccination (12 and 13 months respectively) (supplementary table 1).

Serological analysis was performed at the World Health Organisation reference laboratory for pneumococcal serology, Institute of Child Health, London. Following extraction from whole blood, sera were stored at -70°C prior to assay for pneumococcal serotype-specific immunoglobulin G (IgG) concentrations for the PCV13 pneumococcal serotypes by enzyme-linked immunosorbent assay (ELISA) as previously described.[18] The lower limit of assay quantification was 0.15 µg/mL and IgG concentrations ≥0.35 µg/ml were considered protective.[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 following each vaccination. All AEs (including serious adverse events) were recorded for 28 days after each vaccination using an adverse event (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 prior to and following booster vaccination at 12 months of age; and to quantify the percentage of children experiencing fever, local reactions and non-febrile systemic reactions within 7 days following each vaccine dose.

Pre-trial sample size calculations estimated a minimum of 60 infants in each group to detect at least a 2 fold difference between groups after primary immunization, with 80% power and 5% significance. Based on published data, the standard deviation of IgG responses was estimated be 0.6 $\log_{10}$ units.[20] To allow for drop out 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 using a modified intention to treat analysis including all infants who received a dose of PCV13 and from whom at least one post-vaccination blood sample was obtained. GMCs and 95% confidence intervals (CI) 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 (LLQ) were taken to be half the LLQ 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 using the Student's t-test and the $\chi 2$-test or Fisher's exact test, as appropriate. Statistical significance was defined as $p<0.05$. To facilitate comparisons we have analysed schedules based on the proportions achieving adequate protection for at least half of the serotypes. The number of serotypes with protective concentrations per participant were compared using the non-parametric 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 vaccination, early post-vaccination paracetamol and the presence of chronic lung disease (CLD, 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). Linear regression was not performed on baseline IgG concentrations due to the large number of results below the LLQ.

All data were analyzed using STATA version 13 (Stata Inc).

Results

A total of 210 infants were recruited. 199 participants (94.7%) completed the primary phase (primary endpoint) and 194 (92.4%) completed the entire study (Figure 1). 2 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 prior to their first vaccination (Figure 1).

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

Primary vaccination

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

Following 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) (Figure 2; Supplementary Table 3).

The primary schedule had a significant impact on vaccine immunogenicity. Lack of seroprotection for more than half the PCV13 serotypes was seen in 25%, 12% and 3% of participants receiving the reduced, accelerated and extended schedules respectively ($p<0.001$, supplementary figure 1 and supplementary table 4).

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 (Figure 2, Table 2; Supplementary table 3).

Booster vaccination

At 12 months of age, waning of pneumococcal antibody concentrations was evident with low rates of seroprotection against individual serotypes (Table 3; Supplementary table 5). Antibody concentrations remained significantly higher in those who had received the extended schedule compared with reduced (for 10 serotypes) or accelerated schedules (for 11 serotypes), the accelerated schedule was superior to the reduced schedule for one serotype only.

Following booster vaccination a high proportion of infants achieved protective concentrations (Table 3). As at previous time points, significant variation in antibody concentrations between serotypes and groups was apparent (Figure 3). In contrast to post-primary vaccination responses, participants receiving the extended schedule had lower GMCs compared with the reduced (for 9 serotypes) and accelerated schedules (for 4 serotypes). The accelerated schedule was inferior to the reduced schedule for one serotype (19A) (supplementary table 6). Infants who received the extended schedule had lower fold increases in concentrations following booster vaccination than the other groups (supplementary figure 2).

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 (OR 1.34, 95% CI 1.12-1.60; p=0.001), 14 (OR 1.25, 95% CI 1.12-1.41; p<0.001), 19A (OR 1.27, 95% CI 1.12-1.45; p<0.001) and 19F (OR 1.29, 95% CI 1.09-1.52; p=0.003). 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=0.021), 3 (8% increase per week, 95% CI 4-14, p<0.001) and 7F (8% increase per week, 95% CI 3-13; p=0.002).

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=0.033), 6A (OR 0.26, 95% CI 0.10-0.69; p=0.006), 19A (OR 0.19, 95% CI 0.08-0.45; p<0.001 and 23F (OR 0.23, 95% CI 0.06-0.80, p=0.021). Additionally, 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 regression analysis. An insufficient number of infants (14) received postnatal steroids to analyse any effect. Serotype-specific antibody concentrations after the 12-month PCV13 booster were affected by priming schedule and pre-existing antibody levels only.

Safety and adverse events

There were no significant differences in the frequency or severity of local and systemic AEs between vaccination schedules at any time-point. Altogether 77 serious adverse events (SAEs) were reported (including the 2 deaths). SAEs were predominantly acute respiratory infections. There was 1 possibly related (suspected) unexpected serious adverse reaction

from each randomized group: 2 participants had necrotising enterocolitis within a week of vaccination and 1 participant had post-vaccination cardiorespiratory instability requiring readmission; all 3 infants made a good recovery.

Discussion

This is the first study to compare different PCV13 schedules in premature infants and demonstrates the need for early and effective immunization strategies for this vulnerable group, given their very low pre-immunization 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 immunological 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.[4,21] However, when compared with previous term (PCV13) and preterm (PCV7) studies, antibody concentrations after primary and booster vaccination are lower overall, resulting in lower seroprotection following primary vaccination.[4,5,8,9,22]

Similarly, compared with the recent PCV13 preterm study[7], lower IgG GMCs and seroprotection rates were seen for all serotypes. These differences may be due to the different laboratory testing methodology for serotype-specific antibody concentrations, but potential biological explanations include interactions with concurrently administered vaccines, the younger gestation of our cohort or our broad inclusion criteria encompassing

infants with complex medical problems – representative of the preterm population. Additionally, Martinon-Torres *et al.* did not report baseline IgG concentrations which may differ between countries and impact on post-vaccination concentrations.[7]

When comparing schedules within our 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 following 3 doses (compared with 2 doses) is consistent with two meta-analyses of primary schedules in term infants.[23,24] Of the 3-dose schedules, higher antibody concentrations were seen in premature infants receiving the extended schedule. This 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 as it will allow further maturation of their immune system.[25,26] However, this needs to be set against the optimal age at which protection is required in this population. Several studies have indicated an increased susceptibility of IPD in babies born prematurely when compared with term infants; this risk appears maximal in the first 6 months of life.[1–3]

The differences in response to the booster dose was unexpected as the type of priming schedule has not been consistently shown to affect the generation of immunological memory and PCV booster vaccine 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 thought to be due to lower total antigen exposure favouring 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 one PCV7 priming dose

followed by the 23-valent pneumococcal polysaccharide vaccine (PPV23) at 12 months had higher IgG GMC for serotypes 4, 9V, 19F compared with those who had been primed with two or three PCV7 doses.[13] Similarly, infants receiving a lower antigen-containing investigational tetravalent PCV 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 one serotype.

Despite seroprotective concentrations, infants who had received the extended schedule had lower fold increases in antibody concentrations following booster vaccination than those receiving either the reduced dose or accelerated schedules suggesting that the higher pre-booster antibody concentrations at 12 months may have interfered with booster responses. This effect has been observed following booster doses for other vaccines and several hypotheses have been proposed including the formation of immune complexes consisting of pre-existing 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 our cohort of premature infants, increasing birth gestation was associated with increased immunogenicity. This 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]

Limitations

The study had some potential limitations. The different ages of infants at blood sampling between the groups must be considered when comparing primary schedules; the antibody

concentrations at 7 months for babies in Groups 1 and 2 are not known. It is possible, that infants in those groups may have had a rise in their antibody concentrations between their 5 month sample and 7 months of age due to natural exposure.[40] However, a recent study comparing schedules in term infants which sampled some infants at both 5 and 8 months did not find a rise in antibodies between these ages.[27] We also did not measure antibody concentrations beyond 13 months of age.

As the objectives of this 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 cohort when compared with a recent cohort of term infants in the UK who received a reduced dose schedule, which was analyzed in the same laboratory.[22]

Additionally, we did not include any assessment of functional activity of the antibodies detected. Opsonophagocytic antibody titres 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 PCV schedules in term infants has shown a good relationship between ELISA measured IgG concentrations and opsonophagocytic antibody titres, however an analysis of serotype-specific OPA values did not find a consistent protective OPA titre across all vaccine serotypes.[24,41]

Conclusion

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

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Table 1: Participant characteristics by group.
Median (range) or n (%). CLD: Chronic lung disease. BCG: Bacillus Calmette-Guérin vaccination.

Table 1

	Reduced dose (Group 1) n = 68	Accelerated (Group 2) n = 67	Extended (Group 3) n = 71
Gestation (weeks)	29.6 (24.9-34.9)	30 (23.6-34.9)	30 (23.3-34.9)
Birth weight (g)	1410 (576-2600)	1360 (510-3390)	1390 (450-2680)
Weight at 1st vaccination (g)	2442 (845-4660)	2350 (1260-5070)	2497 (920-4560)
Sex (male)	37 (54)	32 (48)	38 (54)
Ethnicity (white)	57 (84)	54(81)	60 (85)
CLD	23 (34)	22 (33)	27 (38)
Antenatal steroids	59 (87)	56 (84)	62 (87)
Postnatal steroids	4 (6)	4 (6)	6 (8)
Blood transfusion	28 (41)	30 (45)	29 (41)
BCG	5 (7)	5 (7)	7 (10)
Age at visit 1 (days)	61 (49-86)	61 (49-83)	61 (46-88)
Age at visit 2 (days)	93 (78-136)	93 (82-119)	95 (79-132)
Age at visit 3 (days)	126 (111-178)	126 (114-160)	126 (106-160)
Age at visit 4 (days)	158 (132-199)	158 (135-187)	-
Age at visit 5 (days)	-	-	181 (156-258)
Age at visit 6 (days)	-	-	209 (177-298)
Age at visit 7 (days)	368 (353-410)	367 (351-404)	368 (351-429)
Age at visit 8 (days)	400 (367-443)	400 (376-492)	397 (375-606)

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. Proportion (95% CI). a b c: p<0.05 comparing reduced and accelerated, accelerated and extended, and reduced and extended schedules respectively; *p<0.001

Table 2

Serotype	Baseline	Post primary immunization		
	All N = 197	Reduced dose (Group 1) N = 66	Accelerated (Group 2) N = 60	Extended (Group 3) N = 69
1	0.03 (0.01-0.07)	0.85 (0.74-0.92)	0.80 (0.68-0.89)[b]	0.94 (0.86-0.98)
3	0.01 (0.00-0.03)	0.61 (0.48-0.73)	0.66 (0.53-0.78)	0.80 (0.68-0.88)[c]
4	0.02 (0.01-0.05)	0.92 (0.83-0.97)	0.88 (0.77-0.95)	0.94 (0.86-0.98)
5	0.02 (0.01-0.05)	0.36 (0.25-0.49)	0.47 (0.34-0.60)[b]	0.74 (0.62-0.84)[c*]
6A	0.13 (0.09-0.19)	0.58 (0.45-0.70)	0.72 (0.59-0.83)[b*]	0.94 (0.86-0.98)[c*]
6B	0.07 (0.04-0.11)	0.20 (0.11-0.31)[a*]	0.52 (0.38-0.65)[b]	0.78 (0.66-0.87)[c*]
7F	0.05 (0.02-0.09)	0.91 (0.81-0.97)	0.97 (0.88-1.00)	1.00 (0.95-1.00)[c*]
9V	0.06 (0.03-0.10)	0.59 (0.46-0.71)[a]	0.85 (0.73-0.93)	0.93 (0.84-0.98)[c*]
14	0.38 (0.31-0.45)	0.94 (0.85-0.98)	0.98 (0.91-1.00)	0.99 (0.92-1.00)
18C	0.05 (0.02-0.08)	0.88 (0.78-0.95)	0.87 (0.75-0.94)	0.96 (0.88-0.99)
19A	0.24 (0.18-0.30)	0.83 (0.72-0.91)[a]	0.95 (0.86-0.99)	0.96 (0.88-0.99)[c]
19F	0.14 (0.09-0.19)	0.97 (0.89-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
23F	0.06 (0.03-0.10)	0.47 (0.34-0.60)	0.63 (0.50-0.75)[b]	0.83 (0.72-0.91)[c*]

Table 3: Proportion of infants with protective antibody concentrations (IgG ≥0.35 µg/mL) prior to booster vaccination (12 months) and 1 month after booster vaccination. Proportion (95% CI). a b c: p<0.05 comparing reduced and accelerated, accelerated and extended, and reduced and extended schedules respectively; *p<0.001

Table 3

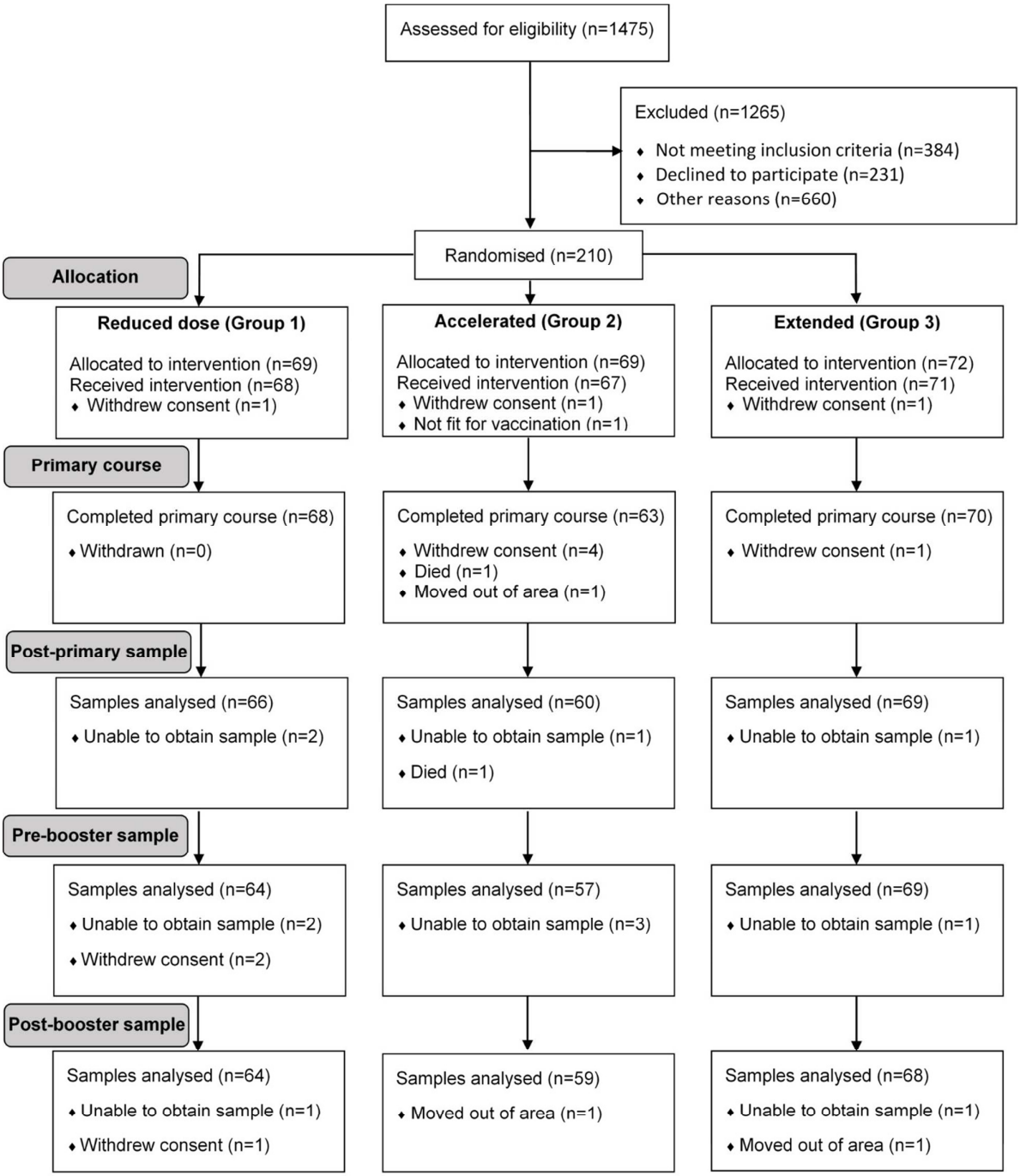
Serotype	Pre-booster vaccination			Post booster vaccination		
	Reduced dose (Group 1) N = 64	Accelerated (Group 2) N =57	Extended (Group 3) N = 69	Reduced dose (Group 1) N = 64	Accelerated (Group 2) N = 59	Extended (Group 3) N = 68
1	0.23 (0.14-0.36)	0.19 (0.10-0.32)^{b*}	0.49 (0.37-0.62)^{c}	0.98 (0.92-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
3	0.18 (0.09-0.30)	0.22 (0.12-0.35)	0.29 (0.18-0.41)	0.89 (0.78-0.95)	0.93 (0.83-0.98)	0.87 (0.76-0.94)
4	0.11 (0.05-0.21)	0.11 (0.04-0.22)^{b}	0.35 (0.24-0.47)^{c*}	1.00 (0.94-1.00)	0.98 (0.91-1.00)	0.99 (0.92-1.00)
5	0.20 (0.11-0.32)	0.14 (0.06-0.26)^{b}	0.32 (0.21-0.44) ^{c*}	0.98 (0.92-1.00)	0.97 (0.88-1.00)	0.93 (0.84-0.98)
6A	0.39 (0.27-0.52)	0.38 (0.25-0.51)^{b*}	0.75 (0.63-0.85) ^{c*}	0.98 (0.92-1.00)	0.98 (0.91-1.00)	1.00 (0.95-1.00)
6B	0.19 (0.10-0.30)	0.16 (0.08-0.28) ^{b*}	0.48 (0.36-0.60) ^{c*}	0.98 (0.91-1.00)	0.97 (0.88-1.00)	0.99 (0.92-1.00)
7F	0.64 (0.51-0.76)	0.68 (0.54-0.80)^{b}	0.86 (0.75-0.93) ^{c}	0.98 (0.92-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
9V	0.06 (0.02-0.15)	0.09 (0.03-0.19) ^{b*}	0.39 (0.27-0.51) ^{c*}	0.98 (0.92-1.00)	0.98 (0.91-1.00)	0.99 (0.92-1.00)
14	0.86 (0.75-0.93)	0.95 (0.85-0.99)	0.99 (0.92-1.00) ^{c}	1.00 (0.94-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
18C	0.06 (0.02-0.15)	0.09 (0.03-0.20) ^{b*}	0.35 (0.24-0.47) ^{c*}	1.00 (0.94-1.00)	0.97 (0.88-1.00)	0.94 (0.86-0.98)
19A	0.39 (0.27-0.53)	0.57 (0.43-0.70)	0.64 (0.51-0.75) ^{c}	1.00 (0.94-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
19F	0.63 (0.50-0.75)	0.49 (0.35-0.63) ^{b*}	0.78 (0.67-0.87)	1.00 (0.94-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
23F	0.15 (0.07-0.26)	0.11 (0.04-0.22) ^{b*}	0.38 (0.27-0.51) ^{c}	0.98 (0.91-1.00)	1.00 (0.94-1.00)	0.97 (0.90-1.00)

Figure 1: Consort diagram

Figure 2: Pneumococcal IgG GMCs following primary vaccination for each serotype and group. a b c: $p<0.05$ comparing groups 1 and 2, 2 and 3, and 1 and 3 respectively. Black capped lines indicate 95% confidence intervals, solid horizontal red line indicates 0.35µg/mL.

Figure 3: Pneumococcal IgG GMCs following booster vaccination for each serotype and group. a b c: $p<0.05$ comparing groups 1 and 2, 2 and 3, and 1 and 3 respectively. Black capped lines indicate 95% confidence intervals, solid horizontal red line indicates 0.35µg/mL.

Figure 1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Excluded (n=1265)
Not meeting inclusion criteria (n=384)
Declined to participate (n=231)
Other reasons (n=660)
Randomised (n=210)
Allocation
Reduced dose (Group 1)
Allocated to intervention (n=69)
Received intervention (n=68)
Withdrew consent (n=1)
Accelerated (Group 2)
Allocated to intervention (n=69)
Received intervention (n=67)
Withdrew consent (n=1)
Not fit for vaccination (n=1)
Extended (Group 3)
Allocated to intervention (n=72)
Received intervention (n=71)
Withdrew consent (n=1)
Primary course
Completed primary course (n=68)
Withdrawn (n=0)
Completed primary course (n=63)
Withdrew consent (n=4)
Died (n=1)
Moved out of area (n=1)
Completed primary course (n=70)
Withdrew consent (n=1)
Post-primary sample
Samples analysed (n=66)
Unable to obtain sample (n=2)
Samples analysed (n=60)
Unable to obtain sample (n=1)
Died (n=1)
Samples analysed (n=69)
Unable to obtain sample (n=1)
Pre-booster sample
Samples analysed (n=64)
Unable to obtain sample (n=2)
Withdrew consent (n=2)
Samples analysed (n=57)
Unable to obtain sample (n=3)
Samples analysed (n=69)
Unable to obtain sample (n=1)
Post-booster sample
Samples analysed (n=64)
Unable to obtain sample (n=1)
Withdrew consent (n=1)
Samples analysed (n=59)
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Samples analysed (n=68)
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Moved out of area (n=1)

Figure 2

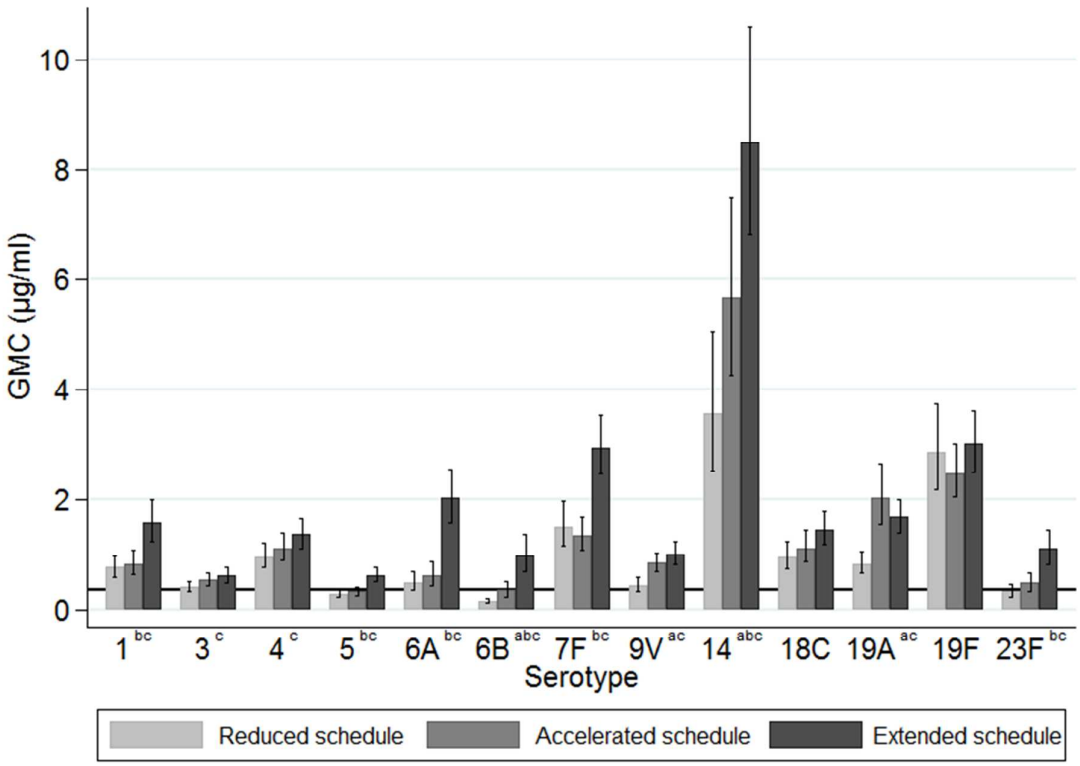
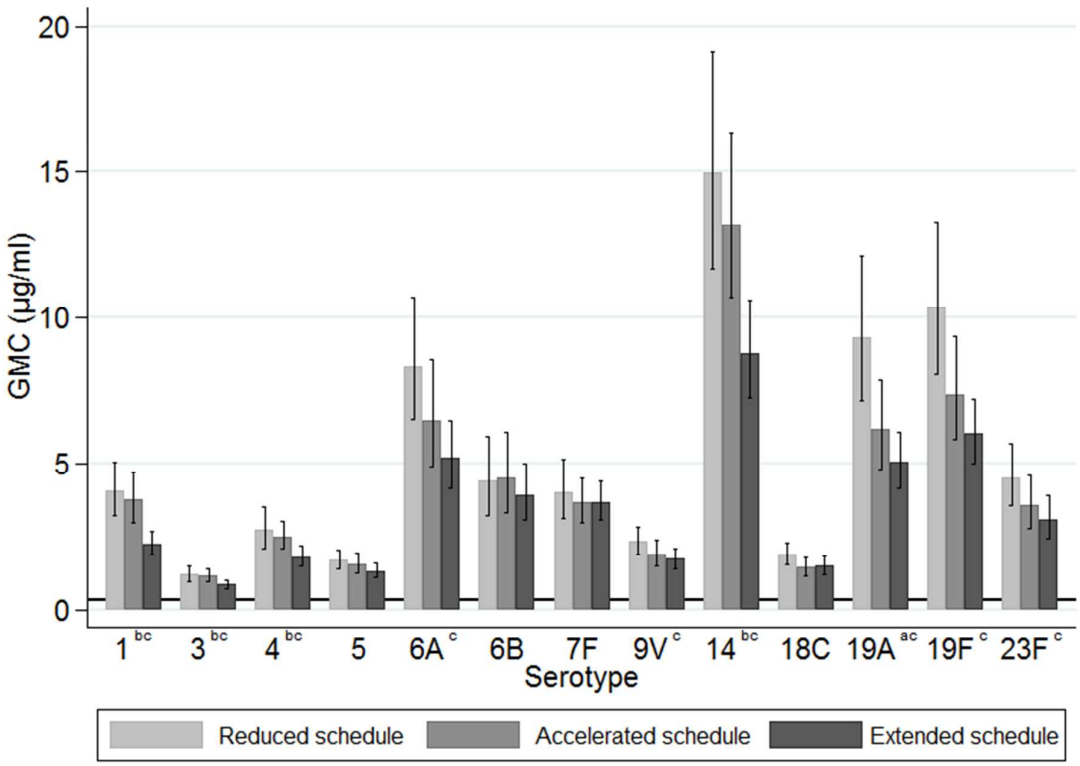


Figure 3



Supplementary tables

Supplementary table 1: Study design. DTaP-IPV-Hib: MenC: Meningococcal serogroup C. V: visit. *blood sample.

Supplementary table 2. Pneumococcal IgG GMCs at baseline (2 months of age) and number of concentrations above the lower limit of quantification for the assay (0.15 µg/mL). There were no significant differences between groups.

Supplementary table 3. IgG GMCs following primary immunization course for each serotype and group. *a b c*: p<0.05 comparing reduced and accelerated, accelerated and extended, and reduced and extended schedules respectively; *p<0.001

Supplementary table 4. Number of serotypes against which individuals attained seroprotection following primary vaccinations. n (%)

Supplementary table 5. IgG GMCs prior to booster vaccination (12 months old) for each serotype by group. *a b c*: p<0.05 comparing reduced and accelerated, accelerated and extended, and reduced and extended schedules respectively; *p<0.001

Supplementary table 6. IgG GMCs following booster vaccination for each serotype by group. a b c: p<0.05 comparing reduced and accelerated, accelerated and extended, and reduced and extended schedules respectively; *p<0.001

Supplementary table 1

Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Age (months)	2	3	4	5	6	7	12	13
Visit window	49-84 days of age	28-42 days after V1	28-42 days after V2	21-42 days after V3	49-70 days after V3	21-42 days after V5	353-390 days of age	21-42 days after V7
Reduced schedule	DTaP-IPV-Hib PCV13 *	DTaP-IPV-Hib MCV	DTaP-IPV-Hib MCV PCV13	*			MMR Hib-MenC PCV13 *	*
Accelerated schedule	DTaP-IPV-Hib PCV13 *	DTaP-IPV-Hib MCV PCV13	DTaP-IPV-Hib MCV PCV13	*			MMR Hib-MenC PCV13 *	*
Extended schedule	DTaP-IPV-Hib PCV13 *	DTaP-IPV-Hib MCV	DTaP-IPV-Hib MCV PCV13		PCV13	*	MMR Hib-MenC PCV13 *	*

Supplementary table 2

Supplementary tables

Serotype	Number > LLQ (%)	IgG GMC (95% CI)		
		Reduced dose (Group 1) N = 68	Accelerated (Group 2) N = 60	Extended (Group 3) N = 69
1	10 (4.9)	0.08 (0.07-0.09)	0.08 (0.07-0.09)	0.08 (0.07-0.09)
3	9 (4.4)	0.08 (0.08-0.09)	0.08 (0.07-0.09)	0.08 (0.07-0.08)
4	13(6.4)	0.09 (0.08-0.10)	0.08 (0.07-0.09)	0.08 (0.07-0.09)
5	13(6.4)	0.09 (0.08-0.10)	0.08 (0.08-0.08)	0.08 (0.08-0.09)
6A	57 (27.6)	0.13 (0.10-0.15)	0.11 (0.09-0.13)	0.12 (0.10-0.15)
6B	45 (22.0)	0.11 (0.09-0.13)	0.10 (0.08-0.11)	0.10 (0.09-0.11)
7F	41 (20.1)	0.11 (0.09-0.13)	0.09 (0.08-0.10)	0.10 (0.09-0.12)
9V	32 (15.7)	0.10 (0.09-0.11)	0.08 (0.08-0.09)	0.10 (0.08-0.11)
14	123 (60.3)	0.29 (0.21-0.40)	0.22 (0.16-0.30)	0.25 (0.18-0.34)
18C	34 (16.7)	0.09 (0.08-0.11)	0.09 (0.08-0.10)	0.10 (0.09-0.12)
19A	116 (56.9)	0.19 (0.15-0.24)	0.17 (0.14-0.22)	0.20 (0.15-0.25)
19F	56 (27.5)	0.13 (0.10-0.16)	0.09 (0.08-0.11)	0.13 (0.10-0.16)
23F	29 (14.2)	0.09 (0.08-0.11)	0.09 (0.08-0.10)	0.10 (0.08-0.11)

Supplementary table 3

Serotype	IgG GMC (95% CI)		
	Reduced dose (Group 1) N = 66	Accelerated (Group 2) N = 60	Extended (Group 3) N = 69
1	0.76 (0.58-0.99)	0.84 (0.64-1.10)[b*]	1.58 (1.25-2.00)[c*]
3	0.42 (0.33-0.53)	0.54 (0.44-0.68)	0.62 (0.50-0.77)[c*]
4	0.97 (0.77-1.22)	1.12 (0.89-1.40)	1.36 (1.12-1.67)[c]
5	0.29 (0.23-0.36)	0.33 (0.25-0.42)[b*]	0.63 (0.50-0.78)
6A	0.49 (0.35-0.69)	0.63 (0.45-0.88)[b*]	2.02 (1.59-2.55)[c*]
6B	0.16 (0.12-0.21)[a*]	0.35 (0.24-0.52)[b*]	0.98 (0.69-1.39)[c*]
7F	1.51 (1.16-1.98)	1.35 (1.08-1.69)[b*]	2.95 (2.46-3.53)[c*]
9V	0.44 (0.34-0.59)[a*]	0.84 (0.68-1.04)	1.02 (0.83-1.25)[c*]
14	3.56 (2.52-5.04)[a]	5.66 (4.27-7.50)[b]	8.49 (6.80-10.60)[c*]
18C	0.96 (0.75-1.23)	1.12 (0.87-1.44)	1.46 (1.20-1.79)[c]
19A	0.84 (0.67-1.05)[a*]	2.03 (1.55-2.64)	1.68 (1.40-2.01)[c*]
19F	2.85 (2.17-3.75)	2.48 (2.04-3.02)	3.00 (2.49-3.62)
23F	0.32 (0.23-0.46)	0.48 (0.34-0.68)[b*]	1.11 (0.84-1.46)[c*]

Supplementary table 4

Number of ST IgG>0.35 µg/mL	Reduced dose (Group 1) N = 66	Accelerated (Group 2) N = 60	Extended (Group 3) N = 69
0	1 (2)	0 (0)	0 (0)
1	0 (0)	1 (2)	0 (0)
2	0 (0)	0 (0)	0 (0)
3	2 (3)	1 (2)	0 (0)
4	2 (3)	1 (2)	1 (1)
5	7 (12)	2 (3)	1 (1)
6	3 (5)	2 (3)	1 (1)
7	3 (5)	3 (5)	0 (0)
8	5 (8)	6 (10)	3 (4)
9	5 (8)	2 (3)	3 (4)
10	6 (10)	5 (9)	3 (4)
11	7 (12)	9 (16)	5 (7)
12	12 (20)	9 (16)	12 (18)
13	7 (12)	17 (29)	38 (57)

Supplementary table 5

Supplementary tables

Serotype	IgG GMC (95% CI)		
	Reduced dose (Group 1) N = 64	Accelerated (Group 2) N = 57	Extended (Group 3) N = 69
1	0.18 (0.14-0.22)	0.18 (0.15-0.22) [b*]	0.36 (0.28-0.45) [c*]
3	0.17 (0.14-0.22)	0.18 (0.14-0.23)	0.22 (0.18-0.27)
4	0.12 (0.10-0.15)	0.13 (0.11-0.16) [b*]	0.24 (0.19-0.29) [c*]
5	0.17 (0.14-0.21)	0.16 (0.13-0.19) [b]	0.25 (0.20-0.30) [c]
6A	0.27 (0.21-0.34)	0.26 (0.20-0.32) [b*]	0.52 (0.41-0.64) [c*]
6B	0.14 (0.11-0.18)	0.17 (0.13-0.21) [b*]	0.31 (0.24-0.39) [c*]
7F	0.44 (0.34-0.56)	0.48 (0.41-0.56) [b*]	0.76 (0.64-0.90) [c*]
9V	0.11 (0.10-0.13)	0.13 (0.11-0.16) [b*]	0.23 (0.19-0.29) [c*]
14	1.02 (0.79-1.32) [a*]	1.63 (1.30-2.05) [b]	2.51 (2.09-3.02) [c*]
18C	0.11 (0.10-0.13)	0.13 (0.11-0.15) [b*]	0.23 (0.19-0.28) [c*]
19A	0.30 (0.22-0.40)	0.32 (0.25-0.40)	0.42 (0.34-0.52)
19F	0.47 (0.38-0.58)	0.38 (0.30-0.47) [b*]	0.61 (0.51-0.72)
23F	0.13 (0.10-0.16)	0.12 (0.10-0.15) [b*]	0.25 (0.20-0.32) [c*]

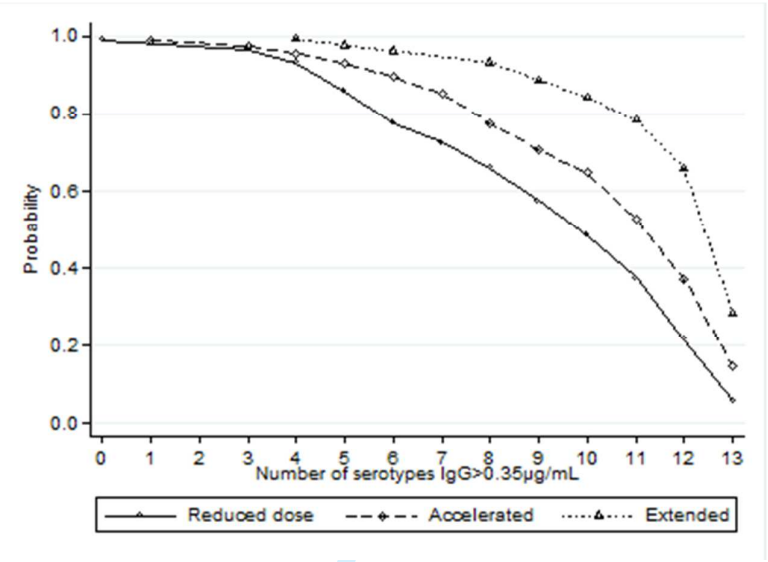
Supplementary table 6

Serotype	IgG GMC (95% CI)		
	Reduced dose (Group 1) N = 64	Accelerated (Group 2) N = 59	Extended (Group 3) N = 68
1	4.05 (3.23-5.07)	3.75 (3.00-4.69) [b*]	2.26 (1.88-2.70) [c*]
3	1.20 (0.97-1.49)	1.18 (0.98-1.43) [b]	0.86 (0.73-1.02) [c]
4	2.74 (2.12-3.55)	2.51 (2.08-3.02) [b]	1.82 (1.52-2.18) [c]
5	1.71 (1.41-2.07)	1.57 (1.27-1.94)	1.33 (1.10-1.62)
6A	8.34 (6.51-10.68)	6.49 (4.91-8.57)	5.21 (4.18-6.48) [c]
6B	4.39 (3.24-5.95)	4.50 (3.34-6.08)	3.91 (3.06-5.00)
7F	4.01 (3.12-5.15)	3.68 (3.01-4.50)	3.70 (3.08-4.43)
9V	2.34 (1.91-2.86)	1.92 (1.53-2.42)	1.73 (1.41-2.12) [c]
14	14.96 (11.70-19.13)	13.21 (10.68-16.33) [b]	8.76 (7.25-10.59) [c*]
18C	1.88 (1.54-2.30)	1.46 (1.17-1.82)	1.50 (1.21-1.87)
19A	9.32 (7.15-12.14) [a]	6.16 (4.80-7.91)	5.03 (4.14-6.10) [c*]
19F	10.36 (8.07-13.31)	7.38 (5.82-9.36)	6.01 (5.02-7.19) [c*]
23F	4.51 (3.56-5.71)	3.58 (2.80-4.58)	3.10 (2.44-3.93) [c]

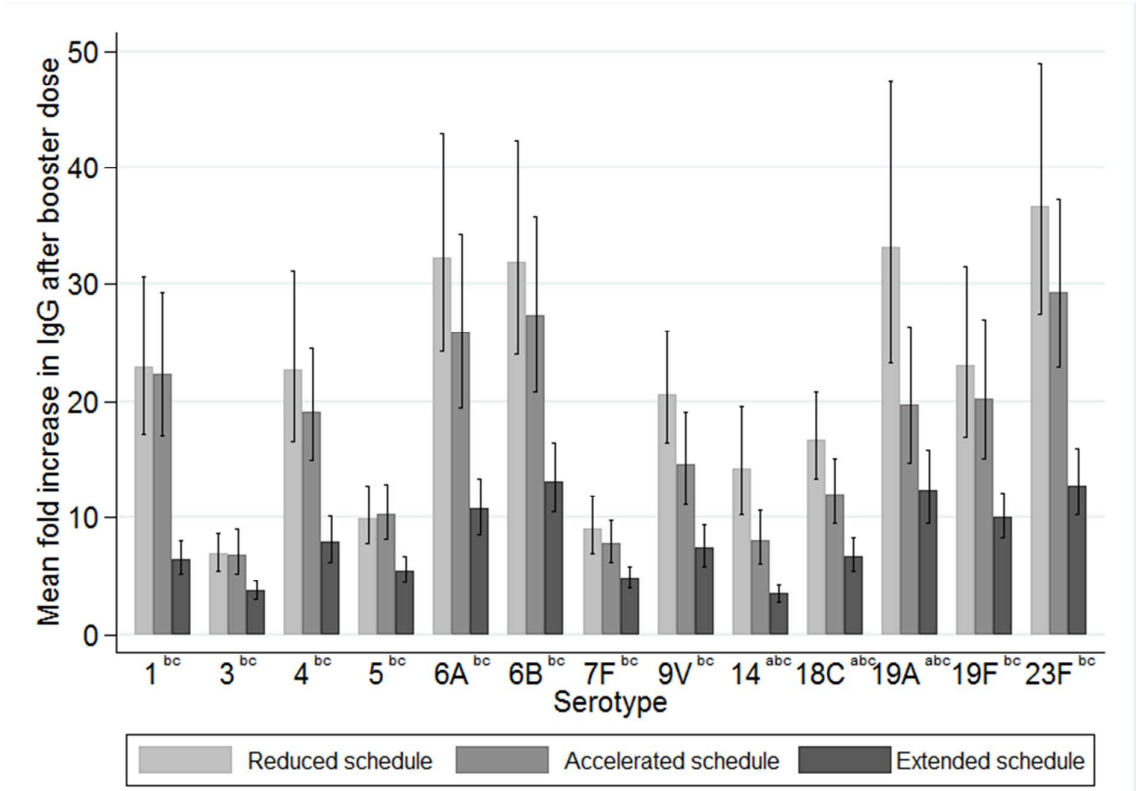
Supplementary figure 1: Reverse cumulative distribution plot of overall seroprotection following primary vaccination for each schedule.

Supplementary figure 2: Fold increases in IgG following booster vaccination for each serotype and group.
Black capped bars indicate 95% CI. a b c: $p<0.05$ comparing reduced and accelerated, accelerated and extended, and reduced and extended schedules respectively.

Supplementary figure 1:



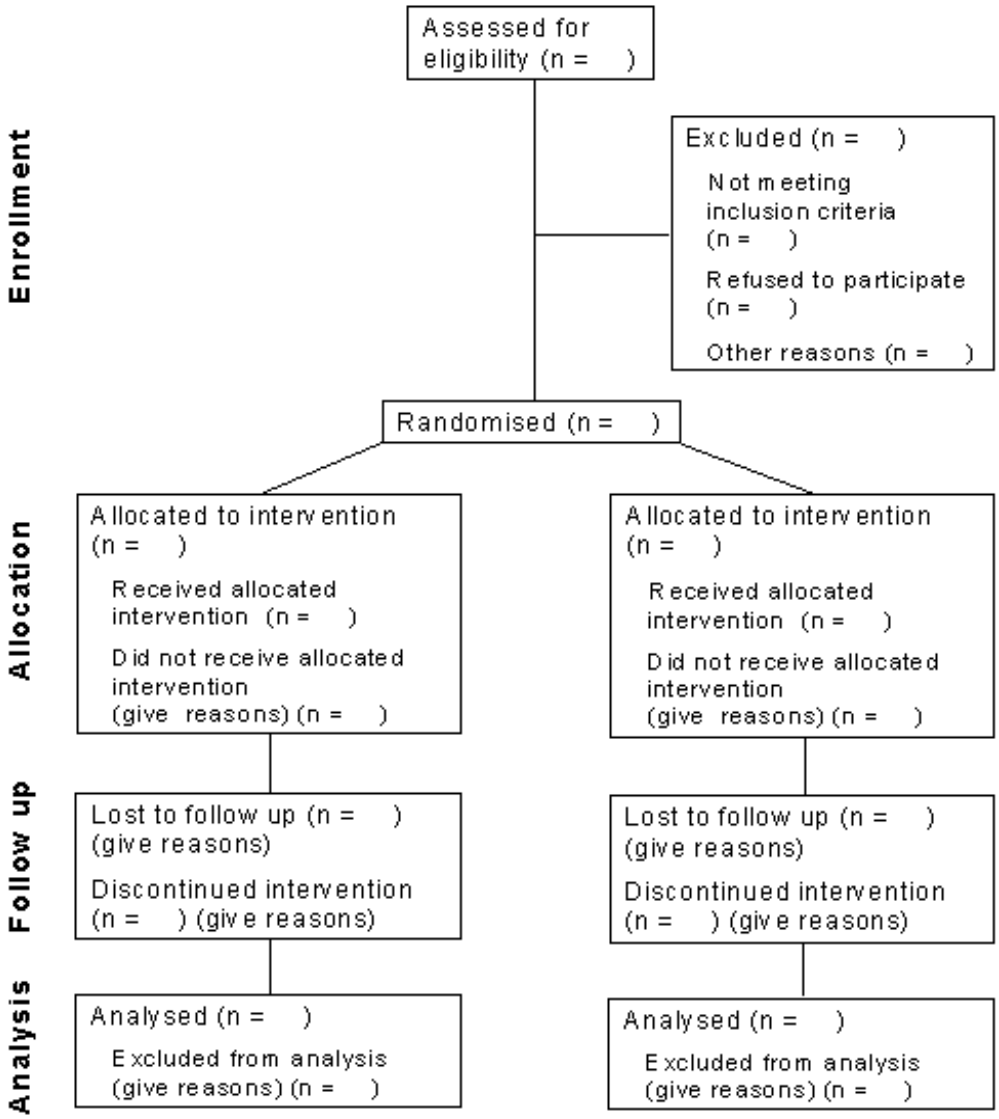
Supplementary figure 2



Checklist of items to include when reporting a randomized trial (56-58)

PAPER SECTION And topic	Item	Description	Reported on page #
TITLE & ABSTRACT	1	How participants were allocated to interventions (*e.g.*, "random allocation", "randomized", or "randomly assigned").	1
INTRODUCTION Background	2	Scientific background and explanation of rationale.	6
METHODS Participants	3	Eligibility criteria for participants and the settings and locations where the data were collected.	7
Interventions	4	Precise details of the interventions intended for each group and how and when they were actually administered.	7-9, sup 1
Objectives	5	Specific objectives and hypotheses.	9
Outcomes	6	Clearly defined primary and secondary outcome measures and, when applicable, any methods used to enhance the quality of measurements (*e.g.*, multiple observations, training of assessors).	9
Sample size	7	How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules.	9
Randomization -- Sequence generation	8	Method used to generate the random allocation sequence, including details of any restriction (*e.g.*, blocking, stratification).	8
Randomization -- Allocation concealment	9	Method used to implement the random allocation sequence (*e.g.*, numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	8
Randomization -- Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	7-8
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. When relevant, how the success of blinding was evaluated.	8
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s); Methods for additional analyses, such as subgroup analyses and adjusted analyses.	9-10
RESULTS Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	fig 1
Recruitment	14	Dates defining the periods of recruitment and follow-up.	7
Baseline data	15	Baseline demographic and clinical characteristics of each group.	tab 1
Numbers analyzed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was by "intention-to-treat" . State the results in absolute numbers when feasible (*e.g.*, 10/20, not 50%).	9, tab 1
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision (*e.g.*, 95% confidence interval).	tab 2,3
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	13
Adverse events	19	All important adverse events or side effects in each intervention group.	13-14
DISCUSSION Interpretation	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	14-16
Generalizability	21	Generalizability (external validity) of the trial findings.	14
Overall evidence	22	General interpretation of the results in the context of current evidence.	14-16

Revised template of the CONSORT diagram showing the flow of participants through each stage of a randomized trial.



~~Optimizing pneumococcal protection in preterm infants: A randomised trial of vaccine schedules~~

Schedules for Pneumococcal Vaccination of Preterm Infants: An RCT

Short title: ~~PCV13 schedules in preterm infants~~**Schedules for Pneumococcal Vaccination of Preterms**

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Dr S Ladhani and Prof P T 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 A J 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 A J Pollard chairs the UK Department of Health's (DH) Joint Committee on Vaccination and Immunization (JCVI); the views expressed in this manuscript do not necessarily reflect the views of JCVI or DH.
Dr S N Faust acts as chief or principal investigators for clinical trials and studies conducted on behalf of 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 SN 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.
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Conflict of Interest Statement:
Dr S Ladhani and Prof P T 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 A J 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 A J Pollard chairs the UK Department of Health's (DH) Joint Committee on Vaccination and Immunization (JCVI); the views expressed in this manuscript do not necessarily reflect the views of JCVI or DH.

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Prof D Goldblatt: Prof D Goldblatt contributes to occasional GSK advisory boards
All other authors have no conflicts of interest relevant to this article to disclose

Abbreviations:

PCV7	7 valent pneumococcal conjugate vaccine
PCV13	13 valent pneumococcal conjugate vaccine
IgG	Immunoglobulin G
GMC	Geometric mean concentrations
IPD	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 to term infants. The optimal primary schedule to generate protective concentrations of pneumococcal antibodies in preterm infants is unknown.

What this study adds:
This 13-valent pneumococcal conjugate vaccine schedule RCT in preterm infants demonstrated that fewer priming doses resulted in higher post-booster, but lower post-primary IgG concentrations. The optimum schedule for preterm infants depends when they are most at risk of invasive disease.

Contributors' Statement Page:

Dr A Kent coordinated the study, performed statistical analysis and drafted the initial manuscript.

Dr S Ladhani assisted with the design of the study, coordination of the study, critically reviewed the manuscript and approved the final manuscript as submitted.

Dr N Andrews approved the data collection tools, performed the statistically analysis, critically reviewed the manuscript and approved the final manuscript as submitted.

Dr T Scorrer, Prof A Pollard, Dr P Clarke, Dr S Hughes, Dr C Heal, Dr E Menson, Dr J Chang, Dr P Satodia, Dr A C Collinson, Dr N Pritchard and Dr S Faust were members of the trial steering committee, recruited participants and were responsible for data collection and study procedures at their sites. They critically reviewed the manuscript and approved the final manuscript as submitted.

Prof D Goldblatt supervised the analysis of all laboratory samples, critically reviewed the manuscript and approved the final manuscript as submitted.

Prof E Miller and Prof P T Heath were responsible for the concept and design of the study and the overall supervision of all aspects of the clinical trial. They critically reviewed the manuscript and approved the final manuscript as submitted.

Abstract

Background

Premature infants have a higher risk of invasive pneumococcal disease and are more likely to have lower vaccine responses compared to term infants. Increasingly, immunization schedules are including a reduced, 2-dose, pneumococcal conjugate vaccine (PCV) priming schedule.

We aimed to assess the immunogenicity of 3 commonly used 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 were measured by ELISA 1 month after primary and booster vaccinations.

Results

A total of 210 infants (median birth gestation 29^{+6} weeks, range 23^{+2}-34^{+6}) were included. Following primary vaccination, 75% (95% CI 62-85), 88% (95% CI 76-95) and 97% (95% CI 87-99) of participants had protective antibody concentrations for at least half the PCV13 serotypes for the reduced, accelerated and extended schedules respectively. Following booster vaccination, participants receiving the extended schedule had significantly lower ($p<0.05$) geometric mean concentrations compared with reduced (for 9/13 serotypes) and accelerated schedules (for 4/13 serotypes).

Conclusions ~~and Relevance~~

Fewer priming doses of PCV13 resulted in lower post-primary concentrations but higher post-booster IgG concentrations~~, but lower post-primary concentrations.~~ than an extended schedule. The optimum vaccine schedule for preterm infants will therefore depend on ~~the setting and~~ when they are most at risk of invasive pneumococcal disease.

Introduction

Premature infants are at increased risk of vaccine preventable diseases, including a two-fold risk of invasive pneumococcal disease (IPD) compared to term infants.[1–3]

In most industrialised countries with established pneumococcal immunization programmes, the 13-valent pneumococcal conjugate vaccine (PCV13) has superseded the 7-valent PCV and has been shown to be highly immunogenic in term infants.[4–6]

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

Additionally, national immunization programmes are increasingly including reduced (2) dose priming schedules ~~due to the diminishing risk of disease in highly vaccinated populations and an increasing number of vaccines to accommodate.~~ [11,12] These schedules are immunogenic in term infants and, with some vaccines, may even improve B cell memory and booster responses.[13–16] However, little is known about the immunogenicity of fewer primary doses in premature infants.

This randomized, controlled trial aimed 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.

Patients and Methods

Participants and recruitment

Premature infants were enrolled in a phase IV open-label randomized controlled trial from 12 UK centres between May 2012 and May 2013. Potentially eligible infants were identified by the clinical teams and parents were provided with information ~~on the trial~~ by the research teams. Infants were eligible for inclusion if they had a birth gestation less than 35^{+0} weeks, had no contra-indications for vaccination as defined by Department of Health guidelines[17] and were between 7 and 12 weeks of age. Additionally, infants should not have received any other vaccinations (with the exceptions of BCG and hepatitis B). Information on the participants' past medical, medication and vaccination history was collected from the medical records using a standardised case report form.

Written informed consent was obtained from parents prior to enrolment. The study was approved by the East of England – Essex research ethics committee (REC reference 07/HO301.11) and registered on the EudraCT clinical trial database (2007-007535-23).

Vaccination

Infants were randomly assigned (1:1:1) to receive PCV13 (Prevenar13; Pfizer, New York) 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)(supplementary table 1). A booster dose of PCV13 was administered to all infants at 12 months of age. Additionally, all participants received a combined diphtheria, tetanus, acellular pertussis, *Haemophilus influenzae* type b and inactivated polio vaccine (Pediacel; Sanofi Pasteur MSD, Lyon, France) at 2, 3 and 4 months old, meningococcal C-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 Hib-MenC-TT conjugate vaccine (Menitorix, GlaxoSmithKline Biologicals, Rixensart, Belgium) at 12 months of age (supplementary table 1). Participants were vaccinated in hospital if still receiving inpatient care. All vaccines were administered intramuscularly.

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

Blood sampling and serological methods

Up to 3 mL of whole blood was obtained from each participant prior to the first vaccination (baseline), 1 month following primary vaccination (at age 5 months for Groups 1 and 2 participants and at age 7 months for Group 3 participants), prior to and 1 month after booster vaccination (12 and 13 months respectively) (supplementary table 1).

Serological analysis was performed at the World Health Organisation reference laboratory for pneumococcal serology, Institute of Child Health, London. Following extraction from whole blood, sera were stored at -70°C prior to assay for pneumococcal serotype-specific immunoglobulin G (IgG) concentrations for the PCV13 pneumococcal serotypes by enzyme-linked immunosorbent assay (ELISA) as previously described.[18] The lower limit of assay quantification was 0.15 µg/mL and IgG concentrations ≥0.35 µg/ml were considered protective.[~~17~~19]

Field Code Changed

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 following each vaccination. All AEs (including serious adverse events) were recorded for 28 days after each vaccination using an adverse event (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 prior to and following booster vaccination at 12 months of age; and to quantify the percentage of children experiencing fever, local reactions and non-febrile systemic reactions within 7 days following each vaccine dose.

Pre-trial sample size calculations estimated a minimum of 60 infants in each group to detect at least a 2 fold difference between groups after primary immunization, with 80% power and 5% significance. Based on published data, the standard deviation of IgG responses was estimated be 0.6 $\log_{10}$ units.[20] To allow for drop out 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 using a modified intention to treat analysis including all infants who received a dose of PCV13 and from whom at least one post-vaccination blood sample was obtained. GMCs and 95% confidence intervals (CI) 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 (LLQ) were taken to be half the LLQ 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 using the Student's t-test and the $\chi 2$-test or Fisher's exact test, as appropriate. Statistical significance was defined as $p<0.05$. To facilitate comparisons we have analysed schedules based on the proportions achieving adequate protection for at least half of the serotypes. The number of serotypes with protective concentrations per participant were compared using the non-parametric 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 vaccination, early post-vaccination paracetamol and the presence of chronic lung disease (CLD, 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). Linear regression was not performed on baseline IgG concentrations due to the large number of results below the LLQ.

All data were analyzed using STATA version 13 (Stata Inc).

Results

A total of 210 infants were recruited. 199 participants (94.7%) completed the primary phase (primary endpoint) and 194 (92.4%) completed the entire study (Figure 1). 2 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 prior to their first vaccination (Figure 1).

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

Primary vaccination

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

Following 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) (Figure 2; Supplementary Table 3).

The primary schedule had a significant impact on vaccine immunogenicity. Lack of seroprotection for more than half the PCV13 serotypes was seen in 25%, 12% and 3% of participants receiving the reduced, accelerated and extended schedules respectively ($p<0.001$, supplementary figure 1 and supplementary table 4).

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 (Figure 2, Table 2; Supplementary table 3).

Booster vaccination

At 12 months of age, waning of pneumococcal antibody concentrations was evident with low rates of seroprotection against individual serotypes (Table 3; Supplementary table 5). Antibody concentrations remained significantly higher in those who had received the extended schedule compared with reduced (for 10 serotypes) or accelerated schedules (for 11 serotypes), the accelerated schedule was superior to the reduced schedule for one serotype only.

Following booster vaccination a high proportion of infants achieved protective concentrations (Table 3). As at previous time points, significant variation in antibody concentrations between serotypes and groups was apparent (Figure 3). In contrast to post-primary vaccination responses, participants receiving the extended schedule had lower GMCs compared with the reduced (for 9 serotypes) and accelerated schedules (for 4 serotypes). The accelerated schedule was inferior to the reduced schedule for one serotype (19A) (supplementary table 6). Infants who received the extended schedule had lower fold increases in concentrations following booster vaccination than the other groups (supplementary figure 2).

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 (OR 1.34, 95% CI 1.12-1.60; p=0.001), 14 (OR 1.25, 95% CI 1.12-1.41; p<0.001), 19A (OR 1.27, 95% CI 1.12-1.45; p<0.001) and 19F (OR 1.29, 95% CI 1.09-1.52; p=0.003). 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=0.021), 3 (8% increase per week, 95% CI 4-14, p<0.001) and 7F (8% increase per week, 95% CI 3-13; p=0.002).

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=0.033), 6A (OR 0.26, 95% CI 0.10-0.69; p=0.006), 19A (OR 0.19, 95% CI 0.08-0.45; p<0.001 and 23F (OR 0.23, 95% CI 0.06-0.80, p=0.021). Additionally, 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 regression analysis. An insufficient number of infants (14) received postnatal steroids to analyse any effect. Serotype-specific antibody concentrations after the 12-month PCV13 booster were affected by priming schedule and pre-existing antibody levels only.

Safety and adverse events

There were no significant differences in the frequency or severity of local and systemic AEs between vaccination schedules at any time-point. Altogether 77 serious adverse events (SAEs) were reported (including the 2 deaths). SAEs were predominantly acute respiratory infections. There was 1 possibly related (suspected) unexpected serious adverse reaction

from each randomized group: 2 participants had necrotising enterocolitis within a week of vaccination and 1 participant had post-vaccination cardiorespiratory instability requiring readmission; all 3 infants made a good recovery.

Discussion

This is the first study to compare different PCV13 schedules in premature infants and demonstrates the need for early and effective immunization strategies for this vulnerable group, given their very low pre-immunization 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 immunological 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.[4,21] However, when compared with previous term (PCV13) and preterm (PCV7) studies, antibody concentrations after primary and booster vaccination are lower overall, resulting in lower seroprotection following primary vaccination.[4,5,8,9,~~20~~22]

Field Code Changed

Similarly, compared with the recent PCV13 preterm study[7], lower IgG GMCs and seroprotection rates were seen for all serotypes. These differences may be due to the different laboratory testing methodology for serotype-specific antibody concentrations, but potential biological explanations include interactions with concurrently administered vaccines, the younger gestation of our cohort or our broad inclusion criteria encompassing

infants with complex medical problems – representative of the preterm population. Additionally, Martinon-Torres *et al.* did not report baseline IgG concentrations which may differ between countries and impact on post-vaccination concentrations.[7]

When comparing schedules within our 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 following 3 doses (compared with 2 doses) is consistent with two meta-analyses of primary schedules in term infants.[23,24] Of the 3-dose schedules, higher antibody concentrations were seen in premature infants receiving the extended schedule. This 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 as it will allow further maturation of their immune system.[25,26] However, this needs to be set against the optimal age at which protection is required in this population. Several studies have indicated an increased susceptibility of IPD in babies born prematurely when compared with term infants; this risk appears maximal in the first 6 months of life.[1–3]

The differences in response to the booster dose was unexpected as the type of priming schedule has not been consistently shown to affect the generation of immunological memory and PCV booster vaccine 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 thought to be due to lower total antigen exposure favouring 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 one PCV7 priming dose

followed by the 23-valent pneumococcal polysaccharide vaccine (PPV23) at 12 months had higher IgG GMC for serotypes 4, 9V, 19F compared with those who had been primed with two or three PCV7 doses.[13] Similarly, infants receiving a lower antigen-containing investigational tetravalent PCV 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 one serotype.

Despite seroprotective concentrations, infants who had received the extended schedule had lower fold increases in antibody concentrations following booster vaccination than those receiving either the reduced dose or accelerated schedules suggesting that the higher pre-booster antibody concentrations at 12 months may have interfered with booster responses. This effect has been observed following booster doses for other vaccines and several hypotheses have been proposed including the formation of immune complexes consisting of pre-existing 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 our cohort of premature infants, increasing birth gestation was associated with increased immunogenicity. This has previously been described for other vaccines and reflects deficiencies in both the innate and adaptive immune systems in these more premature infants.[~~32–37~~34–39]

Field Code Changed

Limitations

The study had some potential limitations. The different ages of infants at blood sampling between the groups must be considered when comparing primary schedules; the antibody

concentrations at 7 months for babies in Groups 1 and 2 are not known. It is possible, that infants in those groups may have had a rise in their antibody concentrations between their 5 month sample and 7 months of age due to natural exposure.[40] However, a recent study comparing schedules in term infants which sampled some infants at both 5 and 8 months did not find a rise in antibodies between these ages.[27] We also did not measure antibody concentrations beyond 13 months of age.

As the objectives of this 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 cohort when compared with a recent cohort of term infants in the UK who received a reduced dose schedule, which was analyzed in the same laboratory.[22]

Additionally, we did not include any assessment of functional activity of the antibodies detected. Opsonophagocytic antibody titres 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 PCV schedules in term infants has shown a good relationship between ELISA measured IgG concentrations and opsonophagocytic antibody titres, however an analysis of serotype-specific OPA values did not find a consistent protective OPA titre across all vaccine serotypes.[~~22,39~~24,41]

Field Code Changed

Conclusion

PCV13 is well tolerated in premature infants ~~but immunogenicity is related to the~~. Different priming ~~schedule and different~~ schedules ~~offer better protection~~result in higher IgG concentrations at different ~~ages. The choice of schedule should therefore reflect the local epidemiology of IPD as the optimal schedule for~~ times during the first 13 months of life. We believe that such data will be of benefit to those planning or providing pneumococcal vaccines to preterm infants ~~depends~~

~~on when they are most at risk. In countries with little IPD in the first year of life a reduced dose primary schedule may be preferred due to higher post-booster antibody concentrations, however the duration of protection remains unknown.~~ and will enable them to consider this in the context of their own immunization programmes and epidemiological situations.

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Table 1: Participant characteristics by group.
Median (range) or n (%). CLD: Chronic lung disease. BCG: Bacillus Calmette-Guérin vaccination.

Table 1

	Reduced dose (Group 1) n = 68	Accelerated (Group 2) n = 67	Extended (Group 3) n = 71
Gestation (weeks)	29.6 (24.9-34.9)	30 (23.6-34.9)	30 (23.3-34.9)
Birth weight (g)	1410 (576-2600)	1360 (510-3390)	1390 (450-2680)
Weight at 1st vaccination (g)	2442 (845-4660)	2350 (1260-5070)	2497 (920-4560)
Sex (male)	37 (54)	32 (48)	38 (54)
Ethnicity (white)	57 (84)	54(81)	60 (85)
CLD	23 (34)	22 (33)	27 (38)
Antenatal steroids	59 (87)	56 (84)	62 (87)
Postnatal steroids	4 (6)	4 (6)	6 (8)
Blood transfusion	28 (41)	30 (45)	29 (41)
BCG	5 (7)	5 (7)	7 (10)
Age at visit 1 (days)	61 (49-86)	61 (49-83)	61 (46-88)
Age at visit 2 (days)	93 (78-136)	93 (82-119)	95 (79-132)
Age at visit 3 (days)	126 (111-178)	126 (114-160)	126 (106-160)
Age at visit 4 (days)	158 (132-199)	158 (135-187)	-
Age at visit 5 (days)	-	-	181 (156-258)
Age at visit 6 (days)	-	-	209 (177-298)
Age at visit 7 (days)	368 (353-410)	367 (351-404)	368 (351-429)
Age at visit 8 (days)	400 (367-443)	400 (376-492)	397 (375-606)

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. Proportion (95% CI). a b c: p<0.05 comparing reduced and accelerated, accelerated and extended, and reduced and extended schedules respectively; *p<0.001

Table 2

Serotype	Baseline	Post primary immunization		
	All N = 197	Reduced dose (Group 1) N = 66	Accelerated (Group 2) N = 60	Extended (Group 3) N = 69
1	0.03 (0.01-0.07)	0.85 (0.74-0.92)	0.80 (0.68-0.89)[b]	0.94 (0.86-0.98)
3	0.01 (0.00-0.03)	0.61 (0.48-0.73)	0.66 (0.53-0.78)	0.80 (0.68-0.88)[c]
4	0.02 (0.01-0.05)	0.92 (0.83-0.97)	0.88 (0.77-0.95)	0.94 (0.86-0.98)
5	0.02 (0.01-0.05)	0.36 (0.25-0.49)	0.47 (0.34-0.60)[b]	0.74 (0.62-0.84)[c*]
6A	0.13 (0.09-0.19)	0.58 (0.45-0.70)	0.72 (0.59-0.83)[b*]	0.94 (0.86-0.98)[c*]
6B	0.07 (0.04-0.11)	0.20 (0.11-0.31)[a*]	0.52 (0.38-0.65)[b]	0.78 (0.66-0.87)[c*]
7F	0.05 (0.02-0.09)	0.91 (0.81-0.97)	0.97 (0.88-1.00)	1.00 (0.95-1.00)[c*]
9V	0.06 (0.03-0.10)	0.59 (0.46-0.71)[a]	0.85 (0.73-0.93)	0.93 (0.84-0.98)[c*]
14	0.38 (0.31-0.45)	0.94 (0.85-0.98)	0.98 (0.91-1.00)	0.99 (0.92-1.00)
18C	0.05 (0.02-0.08)	0.88 (0.78-0.95)	0.87 (0.75-0.94)	0.96 (0.88-0.99)
19A	0.24 (0.18-0.30)	0.83 (0.72-0.91)[a]	0.95 (0.86-0.99)	0.96 (0.88-0.99)[c]
19F	0.14 (0.09-0.19)	0.97 (0.89-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
23F	0.06 (0.03-0.10)	0.47 (0.34-0.60)	0.63 (0.50-0.75)[b]	0.83 (0.72-0.91)[c*]

Table 3: Proportion of infants with protective antibody concentrations (IgG ≥0.35 µg/mL) prior to booster vaccination (12 months) and 1 month after booster vaccination. Proportion (95% CI). a b c: p<0.05 comparing reduced and accelerated, accelerated and extended, and reduced and extended schedules respectively; *p<0.001

Table 3

Serotype	Pre-booster vaccination			Post booster vaccination		
	Reduced dose (Group 1) N = 64	Accelerated (Group 2) N =57	Extended (Group 3) N = 69	Reduced dose (Group 1) N = 64	Accelerated (Group 2) N = 59	Extended (Group 3) N = 68
1	0.23 (0.14-0.36)	0.19 (0.10-0.32)^{b*}	0.49 (0.37-0.62)^{c}	0.98 (0.92-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
3	0.18 (0.09-0.30)	0.22 (0.12-0.35)	0.29 (0.18-0.41)	0.89 (0.78-0.95)	0.93 (0.83-0.98)	0.87 (0.76-0.94)
4	0.11 (0.05-0.21)	0.11 (0.04-0.22)^{b}	0.35 (0.24-0.47)^{c*}	1.00 (0.94-1.00)	0.98 (0.91-1.00)	0.99 (0.92-1.00)
5	0.20 (0.11-0.32)	0.14 (0.06-0.26)^{b}	0.32 (0.21-0.44)^{c*}	0.98 (0.92-1.00)	0.97 (0.88-1.00)	0.93 (0.84-0.98)
6A	0.39 (0.27-0.52)	0.38 (0.25-0.51)^{b*}	0.75 (0.63-0.85)^{c*}	0.98 (0.92-1.00)	0.98 (0.91-1.00)	1.00 (0.95-1.00)
6B	0.19 (0.10-0.30)	0.16 (0.08-0.28)^{b*}	0.48 (0.36-0.60)^{c*}	0.98 (0.91-1.00)	0.97 (0.88-1.00)	0.99 (0.92-1.00)
7F	0.64 (0.51-0.76)	0.68 (0.54-0.80)^{b}	0.86 (0.75-0.93)^{c}	0.98 (0.92-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
9V	0.06 (0.02-0.15)	0.09 (0.03-0.19)^{b*}	0.39 (0.27-0.51)^{c*}	0.98 (0.92-1.00)	0.98 (0.91-1.00)	0.99 (0.92-1.00)
14	0.86 (0.75-0.93)	0.95 (0.85-0.99)	0.99 (0.92-1.00)^{c}	1.00 (0.94-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
18C	0.06 (0.02-0.15)	0.09 (0.03-0.20)^{b*}	0.35 (0.24-0.47)^{c*}	1.00 (0.94-1.00)	0.97 (0.88-1.00)	0.94 (0.86-0.98)
19A	0.39 (0.27-0.53)	0.57 (0.43-0.70)	0.64 (0.51-0.75)^{c}	1.00 (0.94-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
19F	0.63 (0.50-0.75)	0.49 (0.35-0.63)^{b*}	0.78 (0.67-0.87)	1.00 (0.94-1.00)	1.00 (0.94-1.00)	1.00 (0.95-1.00)
23F	0.15 (0.07-0.26)	0.11 (0.04-0.22)^{b*}	0.38 (0.27-0.51)^{c}	0.98 (0.91-1.00)	1.00 (0.94-1.00)	0.97 (0.90-1.00)

Figure 1: Consort diagram

Figure 2: Pneumococcal IgG GMCs following primary vaccination for each serotype and group. a b c: $p<0.05$ comparing groups 1 and 2, 2 and 3, and 1 and 3 respectively. Black capped lines indicate 95% confidence intervals, solid horizontal red line indicates 0.35µg/mL.

Figure 3: Pneumococcal IgG GMCs following booster vaccination for each serotype and group. a b c: $p<0.05$ comparing groups 1 and 2, 2 and 3, and 1 and 3 respectively. Black capped lines indicate 95% confidence intervals, solid horizontal red line indicates 0.35µg/mL.

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2015-3945.R2 – Schedules for Pneumococcal Vaccination of Preterm Infants -- by Kent et al.

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What this study adds. Instead of 'fewer priming doses' should state 'a reduce or accelerated schedule'. Does the last sentence here add anything?	Thank you. We have re-phrased as suggested: "This 13-valent pneumococcal conjugate vaccine schedule RCT in preterm infants demonstrated that a reduced primary schedule resulted in higher post-booster, but lower post-primary IgG concentrations. The optimum schedule for preterm infants depends on when they are most at risk of invasive disease."	Abstract	

Results- third sentence (lines 27-31). The lower booster vaccination results for the extended schedule should be qualified by mentioning that all schedules were adequate post-booster by the study IgG protection criteria.	Thank you, amended text: Following booster vaccination, participants receiving the extended schedule had significantly lower ($p<0.05$) geometric mean concentrations compared with reduced (for 9/13 serotypes) and accelerated schedules (for 4/13 serotypes), but nearly all participations, regardless of schedule or serotype, had seroprotective IgG concentrations.	Abstract	
Conclusion- The first sentence is referencing the reduced schedule, and could be simplified by just stating this.	Amended text: A reduced priming schedule of PCV13 resulted in higher post-booster IgG concentrations, but lower post-primary concentrations	Abstract conclusion	
Conclusion- The second sentence does not add anything here. A more accurate addition would be that IPD threats in the first year of life may be best met by an accelerated or extended schedule.	We have modified slightly to emphasise the lower post primary concentrations first (as this was the primary endpoint of the trial) but have left the 2nd sentence as is to emphasise that these data can now be applied according the relevant setting of the reader: "Fewer priming doses of PCV13 resulted in lower post-primary concentrations but higher post-booster IgG concentrations than an extended schedule. The optimum vaccine schedule for preterm infants will therefore depend on when they are most at risk of invasive pneumococcal disease."	Abstract conclusion	
Introduction- third para (line 33). Are there available statistics on the number or proportion of NIPs using a reduced schedule for PCV13? Also is there a cite available for the stated reasons why NIPs are using reduced schedules?	The World Health Organisation publishes schedules by country. The majority of European, Scandinavian and South American countries use reduced (2) dose schedules. We have therefore added the WHO list as a reference: http://apps.who.int/immunization_monitoring/globalsummary/schedules?sc%5Bd%5D=&sc%5Bv%5D%5B%5D=PNEUMO_CONJ&sc%5BOK%5D=OK. We have also added 2 other references in support of reduced dose schedules: Flasche et al PloS Med 2015; 12:e1001839 Findlow H, Borrow R Hum Vac Immunother 2015; 11: 1501-6	References added	
Methods pg11, line 7. The presented figures and statistics for schedule comparisons are for comparing those with adequate protection for at least half of the serotypes. This analysis standard is not discussed in the methods.	In the methods we state: 'The number of serotypes with protective concentrations per participant were compared using the non-parametric Kruskal–Wallis one-way analysis of variance test. ' We have now added a sentence to the methods: "To facilitate comparisons we have analysed schedules based on the proportions achieving adequate protection for at least half of the serotypes".	**Page 11, line 14**	
Pg15-para1- second sentence. This statement of equal protection is at odds with the post-primary findings and should be revised or removed.	Thank you the comments. We believe the current wording '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 immunological response is dependent on the primary schedule they receive.' highlights that		

	these vaccines are immunogenic in preterm infants but also that the schedule appear to have an effect. We do not believe it implies equal protection between schedules		
Pg 16 line43. The discussion of the relation of priming doses to boosting should be qualified by noting that only one significant difference was observed between the reduced and accelerated schedules after boosting.	We have added the following sentence to emphasize this point 'However, it should be noted that a statistically significant difference between the reduced and accelerated schedule groups was observed for only one serotype.'	Page 17, line 12	
Pg 18 limitations- should also include that no followup testing was done beyond one month past the booster.	Thank you, we have added 'We also did not measure antibody concentrations beyond 13 months of age. '	Page 18, line 12	
The principle objective of the study was stated to be the comparison of protection after the primary series, with the comparison of post-booster results as a secondary objective. This conclusion appears to advocate for the use of the reduced schedule, which seems difficult to support given the findings. All schedules produced adequate post-booster levels; the benefits of the higher booster results from the reduced schedule are thus somewhat speculative without further research. In addition I doubt that many or most NIPs are aware of local IPD epidemiology or of the potential for future outbreaks- and if overall population PCV13 level is factored in, I doubt that there is true differentiation of IPD epidemiology for premature infants on a local level. The authors should reconsider this conclusion statement.	Thank you for your comments. We have tried to avoid supporting one schedule over another as many factors need to be taken into account when implementing a schedule. We have therefore modified the wording so that we are not appearing to advocate one schedule over another. "PCV13 is well tolerated in premature infants but different priming schedules result in higher IgG concentrations at different times during the first 13 months of life. We believe that such data will be of benefit to those planning or providing pneumococcal vaccines to preterm infants and will enable them to consider this in the context of their own immunization programmes and epidemiological situations".	Page 18, line 50	
Supplementary Table 1: Please check the Age row for typos.	Thank you – amended age at visit 3 to 4 months		

Instructions:

Please use this table format to answer the questions posed by the editors and reviewers of your paper. Copy and paste the editor/reviewer's question in the "Comments" column and your answer to that question in the corresponding "Response" column. Be sure to ALSO paste the corrected text along with your response. For minor copyediting changes such as spelling and grammar corrections, you may simply state that the error was corrected, without pasting the altered text.

For clarity, use one row per question. Make sure to list the page and line reference where your change can be found. If no change was made, please make sure to note that in your response in addition to your reasoning. You may delete the sample row and insert rows to this table as needed.