Comparison of two schedules of two-dose priming with the ten-valent pneumococcal conjugate vaccine in Nepalese children: an open-label, randomised non-inferiority controlled trial

Rama Kandasamy, Meeru Gurung, Stephen Thorson, Ly-Mee Yu, Ushma Galal, Merryn Voysey, Sarah Kelly, Brian Wahl, Guy Berbers, Kier Finnegan, Imran Ansari, Krishna Paudel, David R Murdoch, Katherine L O'Brien, Dominic F Kelly, David Goldblatt, Shrijana Shrestha, Andrew J Pollard

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See Comment page 115

Oxford Vaccine Group, Department of Paediatrics (R Kandasamy DPhil, U Galal MSc, M Voysey MBiostat, S Kelly MSc, D F Kelly PhD, Prof A J Pollard FMedSci), **and Nuffield Department of Primary Care Health Sciences** (L-M Yu DPhil, U Galal, M Voysey), **University of Oxford, Oxford, UK; NIHR Oxford Biomedical Research Centre, Oxford, UK** (R Kandasamy, M Voysey, S Kelly, D F Kelly, Prof A J Pollard); **Paediatric Research Unit, Patan Academy of Health Sciences, Kathmandu, Nepal** (M Gurung MD, S Thorson MD, I Ansari MD, Prof S Shrestha MD); **Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA** (B Wahl PhD, K L O'Brien MD); **National Institute for Public Health and the Environment, Bilthoven, Utrecht, Netherlands** (G Berbers PhD); **Great Ormond Street Institute of Child Health, University College London, London, UK** (K Finnegan MSc, Prof D Goldblatt PhD); **Child Health Division, Ministry of Health, Kathmandu, Nepal** (K Paudel MD); **and Department of Pathology, University of Otago, Christchurch, New Zealand** (Prof D R Murdoch MD)

Correspondence to: Dr Rama Kandasamy, Oxford Vaccine Group, CCVTM, Churchill Hospital, Headington, Oxford OX3 7LE, UK
rama.kandasamy@paediatrics.ox.ac.ukw

Summary

Background Nepalese infants receive ten-valent pneumococcal conjugate vaccine (PCV10) with a 1 month interval between priming doses for programmatic reasons. We aimed to investigate whether immune responses to PCV10 serotypes were non-inferior if the second priming dose of PCV10 was delivered at a 1 month interval as opposed to a 2 month interval.

Methods We did an open-label, randomised, parallel group trial in healthy Nepalese infants aged 40–60 days at Patan Hospital, Kathmandu, Nepal. Children were eligible for inclusion if they were healthy, were born at more than or equal to 37 weeks' gestation, were residing in Kathmandu, and had not had any previous vaccinations other than BCG, and oral polio vaccine. Participants were randomly assigned (1:1) by means of a computer-generated list with randomly varying permuted block sizes accessed through a validated web-based interface, to receive PCV10 either at 6 weeks and 10 weeks of age (6+10 group) or at 6 weeks and 14 weeks of age (6+14 group), with both groups receiving a booster at 9 months of age. Laboratory staff, masked to study intervention, analysed serum samples for antibodies against PCV10 serotypes by ELISA. The primary outcome was to determine whether the 6+10 schedule was non-inferior to the 6+14 schedule at 9 months of age, on the basis of the proportion of infants with serotype-specific IgG greater than or equal to 0·35 µg/mL. Non-inferiority was established with a 10% margin, and the primary endpoint was measured in a modified intention-to-treat population, which included only participants who successfully had a blood sample collected. This trial is registered at ClinicalTrials.gov, number NCT02385513.

Findings Between Aug 21, 2015, and April 4, 2016, 304 Nepalese children were randomly assigned to either the 6+10 group (n=152) or the 6+14 group (n=152). At 9 months of age, the 6+10 schedule was non-inferior for serotype 5 (79 [55·2%] of 143 *vs* 78 [53·4%] of 146, difference 1·82% [95% CI −9·6 to 13·25], p=0·021), serotype 9V (66 [46·1%] of 143 *vs* 55 [37·6%] of 146, difference 8·48% [−2·84 to 19·8], p=0·001), serotype 14 (110 [77·4%] of 142 *vs* 110 [74·8%] of 147, difference 2·63% [−7·27 to 12·54], p=0·006), and serotype 19F (135 [95%] of 142 *vs* 146 [100%] of 146, difference −4·92% [−9·86 to 0], p=0·022). At the same timepoint, non-inferiority was not shown for serotype 1 (36 [25·1%] of 143 *vs* 42 [28·5%] of 147, difference −3·39% [95% CI −13·56 to 6·77], p=0·102), serotype 4 (70 [48·9%] of 143 *vs* 87 [59·1%] of 147, difference −10·23% [−21·64 to 1·18], p=0·516), serotype 6B (96 [67·1%] of 143 *vs* 114 [77·5%] of 147, difference −10·41% [−20·65 to −0·18], p=0·532), serotype 7F (99 [69·2%] of 143 *vs* 109 [74·1%] of 147, difference −4·91% [−15·26 to 5·42], p=0·168), serotype 18C (89 [62·2%] of 143 *vs* 114 [77·5%] of 147, difference −15·31% [−25·78 to −4·83], p=0·840), and serotype 23F (37 [25·8%] of 143 *vs* 41 [27·8%] of 147, difference −2·01% [−12·19 to 8·16], p=0·062). After the booster dose, at 10 months of age, there were no significant differences in immunogenicity (proportion of children with antibody greater than or equal to 0.35 µg/mL) for any of the ten serotypes, when comparing the two schedules. Serious adverse events occurred in 32 participants, 11 (7%) of 152 in the 6+10 group and 21 (14%) of 152 in the 6 + 14 group.

Interpretation The 6 week, 14 week, and 9 month schedule should be implemented where possible. However, post-booster responses, which are thought to drive herd immunity, were similar in the two schedules. Therefore, the 6 week, 10 week, and 9 month schedule is an alternative that can be used when logistically necessary, and is expected to provide herd protection.

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Introduction

Streptococcus pneumoniae is the leading cause of bacterial pneumonia, meningitis, and septicaemia in children worldwide, and disproportionately affects children from low-income and middle-income countries.[1] Pneumococcal conjugate vaccines (PCVs) reduce pneumococcal disease

Research in context

Evidence before this study

We searched PubMed on May 1, 2018, for studies comparing the immunogenicity of different pneumococcal conjugate vaccine (PCV) schedules in children using the search terms "immunogenicity" AND "children" AND "PCV" AND "clinical trial" [publication type]. The search was unrestricted by language or publication date. Using this search strategy, we identified one systematic review, which had meta-analysed studies reporting immunogenicity data up until 2011, and two further randomised controlled trials (RCTs) making a head-to-head comparison of PCV schedules, since the systematic review. The previous systematic review examining the effect that timing of seven-valent pneumococcal conjugate vaccine (PCV7) priming schedules had on immunogenicity found that schedules with 2 month intervals between priming doses had improved immunogenicity for three of the PCV7 serotypes before boosting when compared with those with a 1 month interval. Notably there were no RCTs that assessed the interval of PCV priming in a head-to-head design included in this review, with comparisons of intervals made across studies done in different settings. As such, the role of covariates, which have been shown to affect immunogenicity, should be considered. A study completed since this review, compared four 13-valent pneumococcal conjugate vaccine (PCV13) infant schedules (2, 4, and 6 months; 2, 3, and 4 months; 2 and 4 months; or 3 and 5 months) in a head-to-head design among healthy Dutch children, and showed that PCV13 priming schedules with 2 month intervals had improved immunogenicity post-priming when compared with 1 month intervals (2, 4, and 6 months superior for 11 serotypes compared with 2, 3, and 4 months). However, the differences between the schedules diminished with time, with few differences detected after boosting at 11·5 months of age (2, 4, and 6 months superior for two serotypes compared with 2, 3 , and 4 months). Of note, this study was designed in such a way that it could not completely differentiate the interplay between age of initial vaccination and interval of dosing. Another study compared PCV13 administered in three doses at 1 month intervals (2 , 3 , and 4 months) with 2 month intervals (2 , 4, and 6 months) among premature infants in the UK, and showed significantly higher immunogenicity for seven serotypes after the priming series in the children who received PCV13 with 2 month intervals. Children in this study then received a booster at 12 months of age with those children who had PCV13 at 1 month intervals having better immunogenicity for three of the PCV13 serotypes. It should be noted that the findings from this study of premature infants should be translated to healthy infants with caution. It is also difficult to generalise the findings of both of these head-to-head studies, done in European children, which used 11·5–12 months of age boosters, to resource-limited settings where 9-month boosters are used.

Added value of this study

Our study is the first randomised trial to make a head-to-head comparison of a 1 month interval with a 2 month interval PCV priming schedule followed by a 9 month booster. In this trial improved immunogenicity is conferred by a 2 month PCV priming interval; however, the differences between the two schedules lessened over time, particularly after the booster dose.

Implications of all the available evidence

A 2 month interval between priming doses is the preferred strategy for PCV delivery in infants. However, a WHO review, which includes the consideration of data from this trial, indicates that an accelerated priming schedule with a 1 month PCV priming interval might be used where programmatic reasons dictate, since there is little difference between groups post-boosting and there is still a substantial effect expected on invasive disease in resource-limited settings.

burden by direct protection and by reducing naso-pharyngeal carriage, thereby preventing transmission and inducing herd protection.[2,3]

In Nepal, invasive pneumococcal disease is responsible for a substantial disease burden in children.[4,5] Surveillance done since 2005 at Patan Hospital (Kathmandu, Nepal), indicates that the majority of invasive pneumococcal disease is due to serotypes 1, 5, and 14, and that the majority of invasive pneumococcal disease occurs in late infancy and toddlerhood.[5–7] Ten-valent PCV (PCV10) was introduced into the routine infant immunisation schedule of Nepal during 2015.[8]

A randomised trial done at Patan Hospital, assessing the immunogenicity of PCV10, showed that a two-dose prime (at 6 and 14 weeks) with a 9 month booster was non-inferior for IgG concentrations at 18 weeks and 10 months and superior at 2–4 years of age to a conventional three-dose priming-only schedule (6, 10, and 14 weeks).[6] This two-dose prime and boost schedule, with an 8 week interval between the priming doses, is endorsed by WHO and recommended in late 2014 by the Nepal Ministry of Health.[9] WHO however, has also recommended introduction of a single inactivated poliomyelitis virus vaccine at 14 weeks of age to mitigate the risk of outbreaks from vaccine derived serotype 2 poliomyelitis virus, to protect against serotype 2 poliomyelitis virus once countries switch to bivalent oral polio vaccine (OPV; containing only serotypes 1 and 3 poliovirus) from trivalent OPV, and to enhance immunogenicity of OPV to serotypes 1 and 3 poliovirus strains.[10] This approach has created a programmatic dilemma because it requires administering three injections at the 14 week visit (pentavalent vaccine [PCV5], PCV, and inactivated poliomyelitis virus vaccine). On the basis of concerns around public and provider acceptance, and feasibility, the Nepalese Ministry of Health opted to move the second PCV10 priming dose from 14 weeks to 10 weeks

of age, creating a 4 week rather than 8 week interval between the two priming PCV doses.[11] Given this decision, it is important to evaluate the immunogenicity of this accelerated 2+1 schedule (ie, with a 1 month interval between priming doses), comparing it with the standard 2+1 schedule (ie, with a 2 month interval between priming doses), which has been shown to provide a level of immunogenicity that would predict substantial programme effect on disease and colonisation. This immunogenicity is important because accelerated two-dose priming schedules have shown a reduction in immunogenicity in other settings.[12,13]

Therefore, this study was done to evaluate the immunogenicity of a schedule of immunisation with PCV10 at 6 and 10 weeks, which is used in Nepal, compared with priming at 6 and 14 weeks of age in healthy Nepalese infants. In both groups a PCV10 booster was given at 9 months of age.

Methods

Study design and participants

We did a single-centre open-label, parallel group, randomised controlled trial, at Patan Hospital, Kathmandu, Nepal. Ethics approval was obtained from Oxford Tropical Research Ethics Committee (OXTREC 25-15) and the Nepal Health Research Ethics Committee (NHRC 90-2015). A copy of the study protocol can be obtained on request from the corresponding author and is also available online.

For the **R project** see https://www.r-project.org/

For the **study protocol** see https://clinicaltrials.gov/ct2/show/NCT02385513

We recruited healthy Nepalese infants aged 40–60 days, who presented to the immunisation clinic at Patan Hospital. Children were eligible for inclusion if they were healthy, born at more than or equal to 37 weeks' gestation, were residing in Kathmandu, and had not had any previous vaccinations other than BCG and OPV. Children who had any serious condition that might have affected the outcome of the study (for example, a congenital syndrome), had been previously admitted to hospital (except where it was judged not to compromise the study), were born prematurely, had previous immunisation, or had any investigational or non-registered product within 30 days of vaccination were excluded from the trial. Any children with a systemic illness or fever over 38°C at the time of a scheduled study visit had immunisation deferred until they had recovered. Written informed consent was obtained from one parent of all participants. Enrolled children received all other vaccines recommended in the Nepal immunisation programme according to the routine schedule (appendix).

See Online for appendix

Randomisation and masking

After enrolment, participants were randomly assigned by study staff (1:1) to receive PCV10 at either 6 weeks, 10 weeks, and 9 months of age (6+10 group) or at 6 weeks, 14 weeks, and 9 months of age (6+14 group). Participants and clinical staff were not masked to group allocation after the randomisation process was complete; however, all laboratory staff were masked. Randomisation was computer generated, with randomly varying permuted block sizes by means of a fully validated web-based interface system, Sortition.[14]

Procedures

Participants received PCV10 according to their allocated treatment group in addition to their other routine vaccines according to the Nepal vaccination programme schedule. Three blood samples were collected at 14–18 weeks, 9 months, and 10 months of age for analysis of serum antibody responses to the PCV10 serotypes, diphtheria, pertussis, *Haemophilus influenzae* type b (Hib), and tetanus antigens. We collected a nasopharyngeal swab at two timepoints (6 weeks and 10 months of age) to examine carriage of pneumococcus over time and to assess differences between the two groups. At 12 months of age all participants received a dose of varicella-zoster virus vaccine.

Blood was centrifuged for 10 min at 3000 rpm and the serum stored at –20°C or below. Serum samples, masked by study code, were analysed for the ten vaccine serotypes (ie, serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F) by ELISA at the WHO reference laboratory (University College London, London, UK), and for other vaccine antigens (pertussis, diphtheria, tetanus, and Hib) by multiplex immunoassay (Bio-Rad Laboratories, Hercules, CA, USA).[15,16] Nasopharyngeal swabs were collected and processed according to the WHO guidelines.[17] Swabs were placed in a tube of skim-milk-tryptone-glucose-glycerine transport medium and subsequently plated on sheep blood agar. Following overnight incubation at 35–37°C in 5% carbon dioxide, morphologically distinct colonies were selected and subcultured overnight before undergoing serotyping by Quellung reaction.

Outcomes

The primary outcome measure was the percentage of infants with PCV10 serotype specific IgG greater than or equal to 0·35 µg/mL at 9 months of age. Secondary outcomes were: the percentage of infants with PCV10 serotype-specific IgG greater than or equal to 0·35 µg/mL 1 month after the second priming dose of PCV10 and 1 month following the 9 month booster; the geometric mean concentrations (GMCs) of PCV10 serotype specific IgG 1 month following the second priming dose of PCV10, at 9 months of age, and 1 month following the 9 month booster for each of the two study groups; the percentage of infants who experienced an adverse event following the 14 week visit in each of the study groups; and serotype-specific pneumococcal carriage at age 6 weeks, and at age 10 months. Adverse reactions for 7 days following the 14 week visit were solicited by means of a diary card.

Statistical analysis

The pre-specified primary analysis was on a modified intention-to-treat population. That is, if a participant was withdrawn from the study, data collected up to the point of

withdrawal were included in the analysis. For serum IgG values, some participants had a blood sample collected but not all ten serotype-specific antibodies could be measured. In the primary analysis, all available data were included and no imputation of missing data was done. Percentages of participants with IgG greater than or equal to 0·35 µg/mL were calculated for each group with 95% CIs calculated by means of the binomial exact method. For the 9 month visit, the assessment of non-inferiority for each serotype was carried out by means of the Farrington–Manning method with the non-inferiority margin set at 10%.[18] At other timepoints, Fisher's exact test was used to assess differences between the two groups. Serum antibody concentrations were log-transformed and geometric mean con-centrations were compared by calculating the geometric mean ratio. Comparisons between groups were carried out with the Student's *t* test. Fisher's exact test was used to compare the proportion of vaccine type carriage between the two groups. A p value lower than 0·025 (one-sided) was considered to be significant for the non-inferiority analysis of the primary objective, and a p value lower than 0·05 (two-sided) was considered to be significant for other comparisons of superiority. The p values were not adjusted for multiple comparisons.

All analyses were carried out with SAS version 9.4 and R version 3.3.1.[19] Data monitoring was conducted by representatives of the study sponsor.

Sample sizes were based on a previous study in which, at 9 months of age, there were five out of ten serotypes with serotype-specific IgG equal to or higher than 0·2 µg/mL (measured by the GSK ELISA) in at least 93% of participants.[6] We used a WHO reference laboratory ELISA in this study; measures of 0·2 µg/mL in the GSK ELISA correlate with 0·35 µg/mL in the WHO ELISA.

Assuming the same response, 304 participants would provide 90% power (α=0·025) to establish whether the 6+10 schedule was non-inferior to the 6+14 schedule in five out of ten serotypes, and 80% power to establish whether the 6+10 schedule was non-inferior to the 6+14 schedule in seven out of ten serotypes. These calculations allowed for a 10% sample loss (due to laboratory error or loss to follow-up). There was no accounting for multiple comparisons in sample size calculations. This trial is registered at ClinicalTrials.gov, registration number NCT02385513.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had complete access to all study data, did not receive any funding to write this Article, and had final responsibility for the decision to submit for publication.

Results

Between Aug 21, 2015, and April 4, 2016, 850 children were screened and 304 enrolled (figure 1; appendix). 152 children each were randomly assigned to the

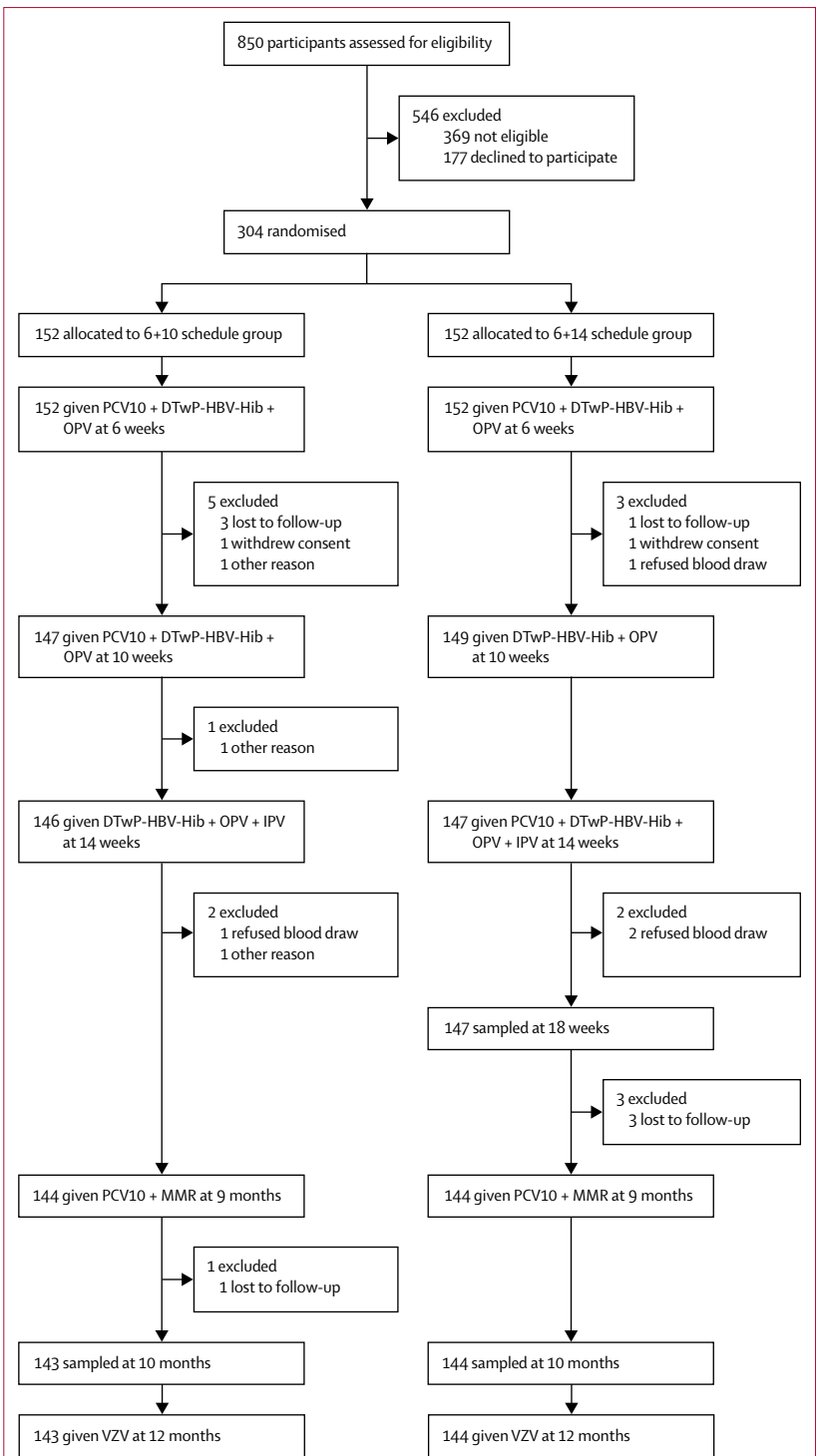

Figure 1: **Trial profile**
PCV10=ten-valent pneumococcal conjugate vaccine. DTwP-HBV-Hib=diphtheria-tetanus-whole cell pertussis-hepatitis B virus-*Haemophilus influenzae* type b vaccine. OPV=oral poliomyelitis vaccine. IPV=inactivated poliomyelitis vaccine. MMR=measles–mumps–rubella vaccine. VZV=Varicella-Zoster virus vaccine.

	6+10 group (n=152)	6+14 group (n=152)	All (n=304)
Mean age at randomisation (weeks)	6·6 (0·4)	6·6 (0·4)	6·6 (0·4)
Mean gestational age (weeks)	39·0 (1·2)	39·3 (1·1)	39·1 (1·1)
Mean birthweight (kg)	3·0 (0·4)	3·1 (0·4)	3·1 (0·4)
Female	74 (49%)	73 (48%)	147 (48%)
Male	78 (51%)	79 (52%)	157 (52%)
Any significant past medical history (hospital admission or general practitioner visit)	22 (14%)	14 (9%)	36 (12%)
Previous vaccination (BCG)	152 (100%)	152 (100%)	304 (100%)
Previous vaccination (HBV)	0	0	0
Previous vaccination (OPV)	2 (1%)	0	2 (1%)
Previous receipt of vitamin A	0	0	0
Taking any medications	18 (12%)	12 (8%)	30 (10%)
Nasopharyngeal swab pneumococcus growth (baseline)	31 (20%)	29 (19%)	60 (20%)

Data are mean (SD) or number of patients (%). HBV=hepatitis B virus. OPV=oral poliomyelitis vaccine.

Table 1: **Baseline characteristics of the intention-to-treat population**

	6 + 10 group		6 + 14 group		Difference, % (95% CI†)	p value for non-inferiority†‡
	n/N*	% (95% CI)	n/N*	% (95% CI)		
Serotype 1	36/143	25·1% (18·2 to 33·1)	42/147	28·5% (21·4 to 36·5)	−3·39% (−13·56 to 6·77)	0·102
Serotype 4	70/143	48·9% (40·5 to 57·4)	87/147	59·1% (50·7 to 67·2)	−10·23% (−21·64 to 1·18)	0·516
Serotype 5	79/143	55·2% (46·7 to 63·5)	78/146	53·4% (44·9 to 61·7)	1·82% (−9·61 to 13·25)	0·021
Serotype 6B	96/143	67·1% (58·7 to 74·7)	114/147	77·5% (69·9 to 84)	−10·41% (−20·65 to -0·18)	0·532
Serotype 7F	99/143	69·2% (60·9 to 76·6)	109/147	74·1% (66·2 to 81)	−4·91% (−15·26 to 5·42)	0·168
Serotype 9V	66/143	46·1% (37·7 to 54·6)	55/146	37·6% (29·7 to 46)	8·48% (−2·84 to 19·8)	0·001
Serotype 14	110/142	77·4% (69·7 to 84)	110/147	74·8% (67 to 81·6)	2·63% (−7·27 to 12·54)	0·006
Serotype 18C	89/143	62·2% (53·7 to 70·2)	114/147	77·5% (69·9 to 84)	−15·31% (−25·78 to −4·83)	0·840
Serotype 19F	135/142	95·0% (90·1 to 97·9)	146/146	100·0% (97·5 to 100)	−4·92% (−9·86 to 0%)	0·022
Serotype 23F	37/143	25·8% (18·9 to 33·8)	41/147	27·8% (20·8 to 35·8)	−2·01% (−12·19 to 8·16)	0·062

*Although a blood sample was collected, for some participants, antibodies to all PCV10 serotypes could not be analysed; as such, the denominators are different for some serotypes. †Score (Farrington–Manning) method. ‡p value <0·025 is considered to be significant.

Table 2: **Summary of participants with IgG ≥0·35 µg/mL at 9 months by serotype (primary analysis)**

6+10 group, or the 6+14 group. Both groups received PCV10 at 9 months of age. The baseline characteristics of the study participants are shown in table 1.

At 9 months of age, the proportion of children achieving serotype-specific IgG greater than or equal to 0·35 µg/mL in the 6+10 group was non-inferior to the proportion of patients in the 6+14 group for serotype 5 (difference 1·82%, 95% CI −9·61 to 13·25; p=0·021), serotype 9V (8·48%, −2·84 to 19·8; p=0·001), serotype 14 (2·63%, −7·27 to 12·54; p=0·006), and serotype 19F (−4·92%, −9·86 to 0; p=0·022). Non-inferiority was not shown for serotypes 1, 4, 6B, 7F, 18C, and 23F (table 2). Sensitivity analysis was done on the per-protocol population by imputing missing data in either a best (IgG assumed to be ≥0·35 µg/mL) or worst (IgG assumed to be <0·35 µg/mL) case scenario. In the best case scenario, non-inferiority was shown for serotypes 5, 9V, 14, and 19F, whereas in the worst case scenario, non-inferiority was shown for serotype 9V (appendix).

1 month after the second priming dose of PCV10, the proportion of participants with IgG greater than or equal to 0·35 µg/mL in the 6+10 group compared with the 6+14 group was significantly lower for serotypes 1, 6B, 18C, and 23F (figure 2 and appendix). At 10 months (1 month following the 9 month booster dose) there were no measurable differences between the groups for serotypes 4, 18C, and 19F since 100% of participants in both groups had IgG greater than or equal to 0·35 µg/mL. For the remaining serotypes (serotypes 1, 5, 6B, 7F, 9V, 14, and 23F) no significant differences were found (figure 2, and appendix).

Geometric mean concentrations of serum IgG at 1 month following the second priming dose were significantly lower in the 6+10 group compared with the 6+14 group for serotypes 6B, 7F, 18C, 19F, and 23F (figure 3, and appendix). At the same timepoint, the geometric mean concentration of serum IgG to serotype 5 was significantly higher in the 6+10 compared with the 6+14 group. At 9 months and

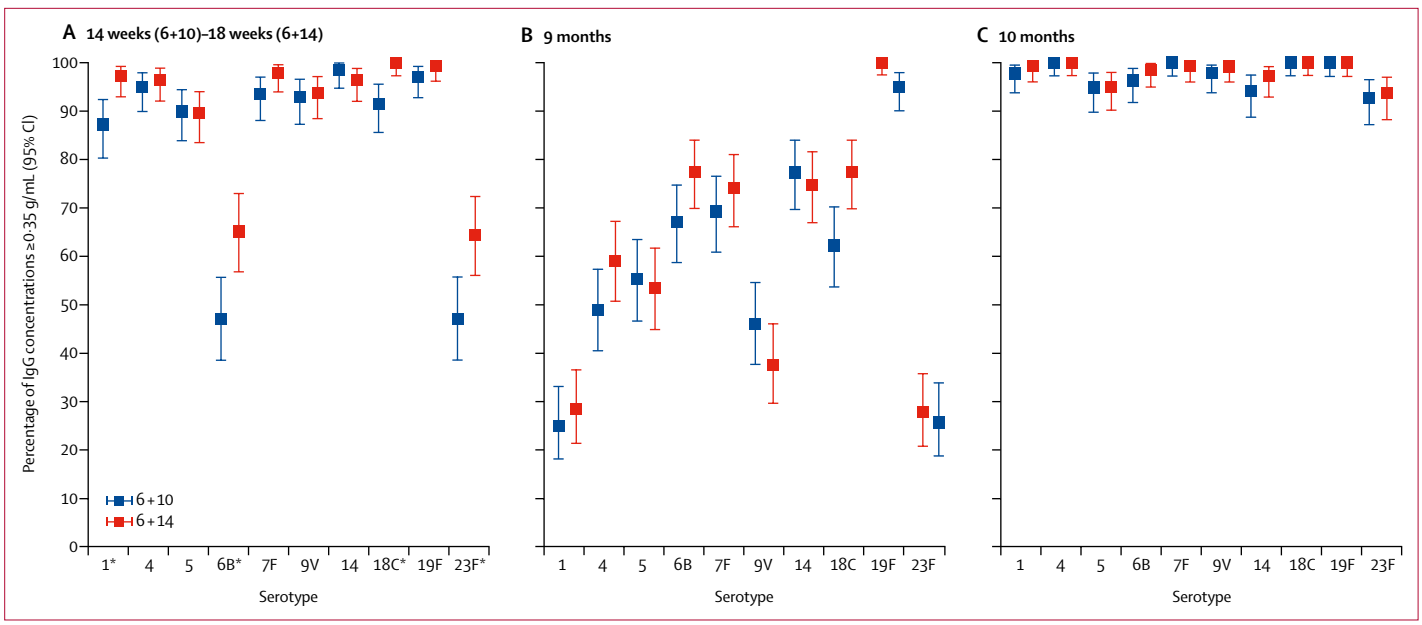

Figure 2: **Proportion of Nepalese children with serum pneumococcal serotype-specific IgG greater than or equal to 0·35 µg/mL**
Sera were collected and analysed at (A) 1 month following the second priming dose (6 + 10 group n=141 and 6 + 14 group n=145), (B) 9 months of age (6 + 10 group n=143 and 6 + 14 group n=147), and (C) 10 months of age (6 + 10 group n=140 and 6 + 14 group n=143). *p<0·05 for 6 + 14 group *vs* 6 + 10 group at 1 month following the second priming dose and 10 months of age.

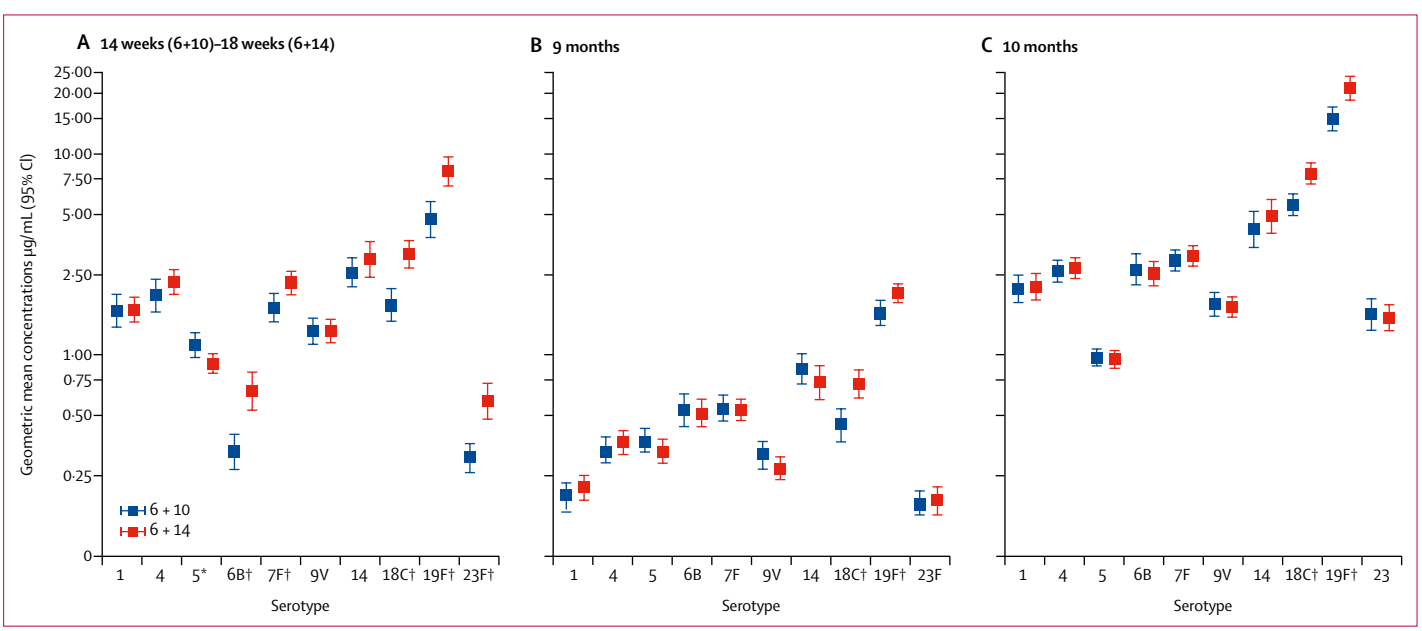

Figure 3: **Geometric mean concentrations of serum pneumococcal serotype-specific IgG among Nepalese children**
Serum samples were collected and analysed at (A) 1 month following the second priming dose (6 + 10 group n=141 and 6 + 14 group n=145), (B) 9 months of age (6 + 10 group n=143 and 6 + 14 group n=147), and (C) 10 months of age (6 + 10 n=140 and 6 + 14 n=143). *p<0·05 for 6 + 10 group *vs* 6 + 14 group.†p<0·05 for 6 + 14 group *vs* 6 + 10 group.

10 months of age geometric mean concentrations were substantially lower in the 6 + 10 group than in the 6 + 14 group for serotypes 18C and 19F (figure 3 and appendix).

Adverse reactions for 7 days following the 14 week visit were solicited by means of a diary card completed by the parents. No significant differences across the 7 day period were seen between the two trial groups for fever, pain, vomiting, diarrhoea, or sleepiness. A significantly higher proportion of children in the 6 + 14 group than in the 6 + 10 group on day 1 had a change in feeding and in crying and irritability (appendix).

	6+10 group	6+14 group	All groups
Participants with an SAE	11/152 (7%)	21/152 (14%)	32/304 (11%)
SAEs related to vaccine receipt	5/13 (39%)	9/24 (38%)	14/37 (38%)
SAEs reported between 6 weeks and 14 weeks (6+10) or 18 weeks (6+14)	11 (7%)	21 (14%)	32 (11%)
SAEs reported between 14 weeks (6+10) or 18 weeks (6+14) and 9 months of age	2 (1%)	2 (1%)	4 (1%)
SAEs reported between 9 and 10 months of age	0 (0%)	1 (1%)	1 (<1%)

Data are number of participants (%) or number of events (%). SAE=serious adverse event.

Table 3: **Summary of serious adverse events that occurred during the trial period**

Nasopharyngeal swabs were collected from participants at 6 weeks and 10 months of age to examine whether any effect on PCV10 carriage could be detected between the trial groups. 60 (20%) of 304 participants had pneumococcus detected at 6 weeks and 170 (59%) of 287 participants had pneumococcus detected at 10 months of age. At 6 weeks of age, five (3%) of 152 children were carrying PCV10 serotypes in the 6+10 group and two (1%) of 152 children were carrying PCV10 serotypes in the 6+14 group. At 10 months of age, 13 (9%) of 143 children in the 6+10 group and 12 (8%) of 144 children in the 6+14 group were carrying a PCV10 serotype (p=0·8375).

Antibody responses to diphtheria, pertussis, Hib b, and tetanus antigens (ie, diphtheria toxin, pertactin, filamentous haemagglutinin, pertussis toxin, Hib, protein D, and tetanus toxin) were assessed at each blood collection timepoint and it was shown that the 6+10 group had lower geometric mean concentrations of IgG than did the 6+14 group to all antigens except filamentous haemagglutinin at 1 month after the second dose of PCV10 (14 weeks in the 6+10 group and 18 weeks in the 6+14 group). At the later timepoints GMCs were similar between the two groups (appendix).

Serious adverse events occurred in 32 participants, 11 (7%) of 152 in the 6+10 group and 21 (14%) of 152 in the 6+14 group (table 3). 14 serious adverse events were deemed to be related to PCV10 administration with five events reported in the 6+10 group and nine events reported in the 6+14 group. No deaths were reported for any of the participants. Medical histories were collected for each of the serious adverse events and are shown in the appendix.

At each study visit, illnesses experienced by the participant since the previous visit were recorded. There was no evidence of difference between the groups in the number of overall illness episodes across the entire study period (appendix). At an illness-specific level there were significantly more cases of pneumonia between the second dose and the booster dose at 9 months of age in the 6+10 group than in the 6+14 group (15 [10%] of 144 in the 6+10 group *vs* five [3%] of 147 in the 6+14 group, p=0·021; appendix). However, when all pneumonia cases before the booster were analysed there was no significant difference between groups (18 [12%] of 147 in the 6+10 group *vs* nine [6%] of 149 and 6+14 group, p=0·071). Across similar time periods, no differences were seen between the groups for reported cases of tonsillitis, upper respiratory tract infections, bronchiolitis, and gastroenteritis (appendix).

Discussion

This is the first trial to compare antibody responses in children who received two doses of PCV10 in early infancy with either a 1 month or 2 month interval between doses, followed by a booster at 9 months of age. Our results show that at 9 months of age, before the booster, the immunogenicity of the 6+10 schedule was non-inferior to the 6+14 group for four of the ten vaccine serotypes (serotypes 5, 9V, 14, and 19F). Non-inferiority was not shown for the other six serotypes (1, 4, 6B, 7F, 18C, and 23F). For these serotypes, the 6+10 group had between 2% and 15% fewer participants with antibody levels achieving the 0·35 µg/mL threshold. At 1 month following the second priming dose of PCV10, the 6+10 group had significantly fewer children with IgG greater than or equal to 0·35 µg/mL for four of the ten serotypes (serotypes 1, 6B, 18C, and 23F). These results indicate that children in the 6+10 group had a period of lower antibodies to six vaccine serotypes in early infancy, before their 9-month booster dose. However, 1 month after receipt of the booster dose, antibody responses were high for all serotypes and there were no significant differences between groups in the proportions of children with IgG greater than or equal to 0·35 µg/mL.

Whether differences in immunogenicity between the study groups in early infancy, before the booster, translate to an increased risk of pneumococcal disease in this community is not known. A re-analysis of a trial of nine-valent pneumococcal conjugate vaccine (PCV9) in The Gambia classified infants according to the spacing of doses received (three doses with 1 month or 2 month intervals), and found no evidence for an increased risk of pneumonia, hospital admission, or mortality in those with the shorter gap between doses.[20] In our study, high antibody responses after the booster dose were achieved for both groups, with at least 92% of children achieving the 0·35 µg/mL threshold for all serotypes, implying no difference in disease risk between groups following the booster during early childhood. In many settings, sustained population-wide PCV use in infancy has induced an indirect effect, protecting the unvaccinated population by interrupting transmission of vaccine serotypes and removing them from circulation.[21] In this region, as in others, carriage of pneumococcus is more prevalent in late infancy and early childhood than in early infancy.[22] As a result, higher postbooster antibody levels in late infancy and their persistence through early childhood are thought to be of

more importance to the interruption of transmission than antibody levels in early infancy. Data from this study supported the WHO advice on pneumococcal vaccine schedules indicating that, although the 6 week, 14 week, and 9 month schedule is preferred, the 6 week, 10 week, and 9 month schedule could be used where programmatic issues demand it.[23] The differences in immunogenicity seen for some serotypes between the 6+10 and 6+14 groups after the second priming dose of PCV10 might translate to only a small effect on vaccine preventable invasive pneumococcal disease risk during the early phases of nationwide vaccine introduction (before establishment of herd protection), after which the timing of infant doses is likely to become of less importance. This notion is exemplified by the fact that some settings with established herd protection are considering the use of one-dose prime and boost (1+1) PCV schedules.[21,24]

The findings from this study are consistent with previous observations showing that administering a PCV at 2 month intervals as opposed to 1 month intervals results in improved immunogenicity.[25,26] For example, a trial that showed a three-dose PCV13 schedule with longer intervals (doses at 2, 4, and 6 months of age) had superior immunogenicity when compared with a three-dose schedule with shorter intervals (doses at 2, 3, and 4 months of age) for nine of the 13 vaccine serotypes 1 month after the priming series.[25] Following boosting these differences diminished, with the 2, 4, and 6 month schedule superior to the 2, 3, and 4 month schedule for GMCs of IgG to serotypes 18C and 23F only.

Examination of immunogenicity by use of GMCs showed similar observations to those made when comparing the groups by use of the 0·35 µg/mL threshold. Interestingly, GMCs for serotypes 18C and 19F were higher in the 6+14 compared with the 6+10 group at 9 months and 10 months, which might be related to the difference in interval between priming doses, or age maturation of the immune system differing between the two groups when the second PCV10 dose is given. Notably, the pneumococcal polysaccharides for serotypes 18C contained within PCV10 are conjugated to tetanus toxoid and for 19F are conjugated to diphtheria toxoid. In this study, PCV5 which contains tetanus and diphtheria toxoids was administered at 6, 10, and 14 weeks of age. As such, it might also be that the extra dose of PCV5 that the 6+14 group receive before the second dose of PCV10 has a role in augmenting the immunogenicity to serotypes 18C and 19F.

Between the second dose and the 9 month booster, children in the 6+10 group had significantly more cases of pneumonia than did children in the 6+14 group. This statistic might be a chance finding, or might be potentially due to the 4 week longer observation period in the 6+10 group for this analysis. In addition, this study was not designed to measure the differences in pneumonia cases in a standardised way. It is unknown whether the cases of pneumonia in this study were due to PCV10 covered serotypes, non-PCV10 serotypes, other bacteria, or viruses. Larger epidemiological studies, specifically designed to measure pneumonia prevalence among children receiving different PCV schedules would be better suited to identifying any difference in the risk of developing pneumonia as a result of vaccine timing.

The strengths of this trial are its randomised design, careful timing of sample collection allowing valid comparisons across groups, sample size powered for the primary outcome, low prevalence of loss to follow-up, and use of a standardised assay to assess the primary outcome. In this trial, timing of vaccine administration was strictly adhered to; however, it is important to consider the effect of vaccine adherence in the general population, and how transferable findings in the trial are to those receiving the vaccine in the community. In Nepal the trial findings are likely to correlate well with observations in the community with WHO estimating coverage of the third PCV5 (at 14 weeks of age) to be 87% in 2016,[27] whereas one study reports adherence to the Expanded Program on Immunization for delivery of the first PCV5 dose (at 6 weeks of age) to be almost 80%.[27,28] How well the community will adhere to the 6+14 or the 6+10 PCV10 schedule is difficult to predict from this trial alone, and further study into community and health-care provider preferences are needed to address this question.

Limitations of this trial include the open-label design, the timeframe of blood sampling, multiple comparisons, and single-centre design. Since participants and staff were aware of study arm allocation, there is the possibility that this knowledge influenced the reporting of any safety signals. Blood sampling following the second PCV priming dose was done at 14 weeks of age in the 6+10 group and 18 weeks of age in the 6+14 group, and, as such, there is a 1 month age difference between the groups when comparing this timepoint, which is not the case with the 9 month and 10 month timepoints. There were multiple timepoints and multiple serotypes included in the analysis. The study was powered only on the primary outcome and not powered to account for multiple comparisons and therefore only unadjusted p values are presented. It is therefore possible that some findings are due to chance alone and should be interpreted with caution. The single-centre design means that careful consideration should be given when translating the findings to other settings. Should similar studies be done in the future, meta-analysis of the unadjusted data reported in this study might provide more robust results.

Overall this trial shows that the safety profile of the 6+10 and 6+14 PCV10 schedules were similar; however, improved immunogenicity in early infancy was seen for the 6+14 group. Direct protection might be reduced in early infancy among children receiving PCV10 at 6 weeks and 10 weeks during the course of vaccine introduction. However, the interruption of transmission and creation of herd protection through high-coverage, nationwide implementation of PCV10 is likely to ameliorate progressively any difference in disease risk owing to lower

immunogenicity in early infancy for the 6+10 schedule. As such, the data reported in this trial should not impair flexibility in programmatic implementation of either schedule, as advised in WHO recommendations.[23,29]

Contributors

RK designed the study, coordinated study implementation, analysed the data, and led the writing of the report. MG, ST, and BW designed the study and coordinated local study implementation. L-MY and UG did the statistical analysis. MV, IA, KP, DRM, KLO, DFK, and SS designed the study. SK designed the study and coordinated study implementation. GB led the analysis of serum for antibodies to PCV5 antigens. KF did analysis of serum for anti-pneumococcal antibodies. DG led the analysis of serum for anti-pneumococcal antibodies. AJP designed the study and coordinated study implementation. All authors contributed to the interpretation of data and subsequent writing, reviewing, and revision of the manuscript.

Declaration of interests

AJP reports grants from Okairos and Pfizer, which finished within the past 36 months outside the submitted work. AJP is Chair of UK Department of Health's Joint Committee on Vaccination and Immunisation, and Chair of the EMA Scientific Advisory Group on vaccine; however, the views expressed herein do not represent necessarily those of the Department of Health or the Joint Committee on Vaccination and Immunisation. AJP is also a member of WHO's Strategic Advisory Group of Experts. DFK receives salary support from the National Institute for Health Research Oxford Biomedical Research Centre. RK received the Robert Austrian Award in Pneumococcal Vaccinology, which was supported by Pfizer, at the 10th International Symposium on Pneumococci and Pneumococcal Diseases 2016. KLO has had grant funding in the preceding 3 years from Pfizer, the Bill & Melinda Gates Foundation and Gavi for pneumococcal vaccine related research. DG reports grants from vaccine manufacturers GlaxoSmithKline, Sanofi Pasteur, and Merck, outside the submitted work.

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