Optimising pneumococcal protection in preterm infants:
A randomised controlled trial of 3 primary schedules.

Authors:
Kent, Alison (1)
Andrews, Nick J(2)
Scorrer, Tim (3)
Pollard, Andrew J (4)
Snape, Matthew D (4)
Clarke, Paul (5)
Hughes, Stephen (6)
Heal, Carrie (7)
Menson, Esse (8)
Chang, John (9)
Satodia, Prakash (10)
Collison, Andrew C (11)
Prichard, Nicola (12)
Faust, Saul N (13)
Ladhani, Shamez N (2)
Miller, Elizabeth (2)
Goldblatt, David (14)
Heath, Paul T (1)

Author Affiliations:
1. Paediatric Infectious Diseases Research Group and Vaccine Institute, St George's, University of London, London, UK
2. Immunisation, Hepatitis and Blood Safety Department, Public Health England, Colindale, London, UK
3. Neonatal Unit, Queen Alexandra Hospital, Portsmouth, UK
4. Oxford Vaccine Group, University of Oxford, and the NIHR Oxford Biomedical Research Centre, Oxford UK
5. Neonatal Unit, Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, UK
6. Department of Immunology, Royal Manchester Children's Hospital, Manchester, UK
7. Neonatal Unit, Stepping Hill Hospital, Stockport, UK
8. Department of Paediatric Infectious Diseases, Evelina London Children's Hospital, London, UK
9. Neonatal Unit, Croydon University Hospital, London, UK
10. Neonatal Unit, University Hospital Coventry, Coventry, UK
11. Neonatal Unit, Royal Cornwall Hospital, Truro, UK
12. Neonatal Unit, Royal Berkshire Hospital, Reading, UK
13. NIHR Wellcome Trust Clinical Research Facility, University of Southampton, Southampton
14. Institute of Child Health, UCL, London, UK

Short Title: Optimising pneumococcal protection in preterm infants

Abbreviations:

PCV7	7 valent pneumococcal conjugate vaccine
PCV13	13 valent pneumococcal conjugate vaccine
DTaP-IPV-Hib	Diphtheria, tetanus, acellular pertussis, inactivated poliovirus and Haemophilus influenzae type b vaccine
MCV	Meningococcal conjugate vaccine
IgG	Immunoglobulin G
GMC	Geometric mean concentrations
IPD	Invasive pneumococcal disease

Key words:
Infant, premature
Pneumoccocal vaccines
Immunization schedule
Gestational age
Immunoglobulin G

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What's known on this subject:
Premature infants have increased risk of invasive pneumococcal disease and diminished vaccine responses compared to term infants. Reduced dose PCV schedules do not significantly impact on vaccine protection in term infants. Their immunogenicity in preterm infants is not known.

What this study adds:
In premature infants, reduced dose PCV schedules provide less protection after primary vaccination. A 2, 4 and 6 month schedule was most immunogenic. Conversely, following booster vaccination, highest antibody concentrations were seen in those receiving a reduced dose priming schedule.

Abstract

Background and objectives
In term infants, reduced dose PCV schedules do not significantly impact on seroprotection rates. Premature infants have increased incidence of invasive pneumococcal disease and lower antibody concentrations following PCV7 compared to term infants. As a result of serotype replacement, the use of PCV13 has largely superseded PCV7. We aimed to assess the immunogenicity of 3 priming schedules commonly used in national immunisation programmes, including after a booster dose at 12 months of age, in premature infants

Methods
210 infants (<35 weeks gestation) were randomised to receive PCV13 at 2 & 4 months (reduced dose schedule), 2, 3 & 4 months (accelerated schedule) or 2, 4 & 6 months (extended schedule). All infants received a booster dose at 12 months. Pneumococcal IgGs for the PCV13 serotypes were measured prior to and 1 month following the primary and booster vaccinations.

Results

The median gestational age was 29^{+6} weeks (range 23^{+2}-34^{+6}). Following primary vaccination, increased antibody concentrations were seen for all serotypes regardless of schedule. Participants who had received the extended schedule had significantly higher IgG GMCs compared to the reduced dose (11 serotypes) and accelerated schedules (7 serotypes). The accelerated schedule was superior to the reduced dose schedule for 4 serotypes. Conversely, following booster vaccination, the extended schedule group had significantly lower GMCs compared to the reduce dose schedule (9 serotypes) and accelerated schedule (4 serotypes).

Conclusions
PCV13 immunogenicity in preterm infants is related to priming schedule and different schedules offer protection at different ages. The optimum schedule for preterm infants therefore depends on when they are most at risk of invasive disease.

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Introduction

Premature infants are at increased risk of vaccine preventable diseases including invasive pneumococcal disease (IPD) compared to term infants and the dramatic fall in IPD following the widespread introduction of pneumococcal conjugate vaccines (PCV) has not addressed this problem[1–5]. A likely contributing factor is the reduced antibody concentrations for the 7 pneumococcal serotypes (ST) included in PCV7 following primary and booster immunisations, due to the relative immaturity of the preterm infants' immune system[6–8]. Following concerns about serotype replacement, PCV13 (containing 6 additional ST) has largely replaced PCV7 and has been shown to be highly effective in term infants but it's immunogenicity in preterm infants has not been described[9–11].

National immunisation schedules increasingly include reduced-dose priming schedules due to the diminishing risk of disease in highly vaccinated populations and an increasing number of vaccines to accommodate. These schedules are immunogenic in term infants and, in certain cases, may actually improve B cell memory and booster responses[12–14]. However, little is known about the ability of the preterm infant's immune system to respond to fewer primary doses. Additionally, full 3 dose schedules vary in the timing of doses; 'accelerated' schedules aim to provide protection early in life with minimum intervals between doses (e.g. doses administered at 2, 3 and 4 months of age), whilst 'extended' schedules are spread over a longer period (e.g. 2, 4 and 6 months of age) to benefit from increased immune system maturity and less inhibition from transplacentally-acquired maternal antibodies[15,16].

Whilst low levels of maternal antibody at birth may leave premature infants vulnerable to infection in the immediate postnatal period, higher levels of maternal antibody have been associated with a poorer immune response to vaccination and thus (in theory) these low levels

may allow higher antibody concentrations to be achieved following vaccination. We aimed to assess the immunogenicity and protection afforded by reduced dose, accelerated and extended PCV13 priming schedules commonly used in national immunisation programmes of premature infants, including after a booster dose at 12 months of age.

Patients and Methods

Participants and recruitment

210 premature infants were enrolled into a phase IV open-label randomised controlled trial from 12 UK centres between May 2011 and May 2013.

Potentially eligible infants were identified by the clinical team and parents were provided with information on the trial by the research team. Infants were eligible for inclusion if they had a birth gestation less than 35^{+0} weeks, were clinically fit for vaccination as defined by Department of Health guidelines[17] and were between 7 and 12 weeks of age. Additionally, infants had to have not received any primary vaccinations (excluding BCG and hepatitis B) and be able to attend appointments or be visited at home for the duration of the study. Receipt of blood products or postnatal steroids were not an exclusion criteria. Recruitment was stratified with half of participants born at less than 30^{+0} weeks gestation.

Written informed consent was obtained from parents or legal guardians prior to recruitment. The study was approved by the East of England – Essex research ethics committee (REC reference 07/HO301.11)

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). 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 (DTaP-Hib-IPV) vaccine (Pediacel; Sanofi Pasteur MSD, Lyon, France) at 2, 3 and 4 months old, meningococcal C-CRM_{197} vaccine (MCV) (Menjugate; Novartis Vaccines, Siena, Italy) at 3 and 4 months of age and a combined measles, mumps and rubella (MMR) vaccine (Priorix; GlaxoSmithKlein Biologicals, Rixensart, Belgium) and Hib-MenC-TT conjugate vaccine (Menitorix, GlaxoSmithKlein Biologicals, Rixensart, Belgium) at 12 months of age. All vaccines were administered intramuscularly and where two vaccines were administered concurrently in the same limb, they were injected at least 2.5cm apart.

Computerised block randomisation 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 envelope containing the group allocation opened. The study was not blinded.

Blood sampling and serological methods

Up to 3mls of whole blood were obtained from each participant prior to the first immunisation (baseline), one month following primary vaccination (at 5 months of age for participants receiving the reduced dose or accelerated schedule and at 7 months of age for those receiving the extended schedule), prior to booster vaccination and one month following booster vaccination (12 and 13 month old respectively).

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[18,19]. The lower limit of quantification for the assay was 0.15μg/mL and IgG concentrations ≥0.35μg/ml were considered protective[20].

Safety analysis

All participants were observed for immediate adverse reactions including anaphylaxis. Solicited systemic and local adverse reactions were recorded by the infant's main caregiver (parent or, if an inpatient, nursing staff) for 7 days following each vaccination. All adverse events (AE) were recorded for 28 days after each vaccination. In addition, participants who were inpatients at the time of vaccination had continuous cardiorespiratory monitoring and details of any cardiorespiratory instability and ventilatory support required prior to and following vaccination were recorded.

Statistical analysis

The primary objective of this trial was to assess differences in IgG geometric mean concentrations (GMC) and the proportion of infants with protective antibody concentrations between schedules one month after completion of the primary vaccination course. The main secondary objectives were to assess between-schedules differences in IgG GMC and seroprotection rates 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 the 7 days following each vaccine dose.

A comparison of schedules was estimated to require 60 infants in each group at the post-primary blood sample to detect 2.10 fold differences between groups to be detectable with 80% power at 5% significance. Based on data from recent studies, the standard deviation of

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IgG responses was expected to be around 0.6 $\log_{10}$ units. To allow for 15% drop out of subjects over the course of the study and the challenges of obtaining blood samples from very premature infants, 210 infants were recruited.

Data were analysed on a modified intention to treat analysis including all infants vaccinated with at least one post vaccination blood sample. GMCs and confidence intervals (CI) were calculated for each sampling time point, along with the proportion of infants achieving protective antibody concentration and binominal CIs. Results below the lower limit of quantification were taken to be half the LLQ for computational purposes.

Statistical comparison of antibody concentrations between the 3 trial arms were performed using the students T-test and proportions above the pre-defined protective thresholds compared using the χ^2-test or Fisher's Exact Test, as appropriate.

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

Results

210 infants were recruited with a median birth gestation of 29^{+6} (range 23^{+2}-34^{+6}). A number of infants did not meet the inclusion criteria; the majority of these infants were not in the appropriate age range or were not stable enough for vaccination. A second, larger, group of infants were excluded for logistical reasons (Figure 1). Due to the nature of neonatal intensive care in the UK, infants may not be hospitalised for near their home and therefore many infants were transferred back to their local neonatal service prior to their first vaccination and families were unable to travel for follow-up study visits.

The baseline characteristics of randomised infants were similar between groups (Table 1).

199 participants completed the primary phase (primary endpoint) and 194 completed the entire study (Figure 1). 2 participants died unexpectedly at home, both deaths were not

related to the trial (bronchopneumonia and Group B Streptococcus septicaemia with meningitis).

Primary vaccination

Prior to their first vaccine, participants had very low antibody concentrations for all serotypes, with a high proportion of concentrations below the LLQ of the assay (supplementary table 1). The highest IgG GMCs were seen for serotype 14 and 19A (0.26µg/mL and 0.19µg/mL respectively). The proportion of infants with protective antibody concentrations was low and did not vary between groups (Table 2).

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

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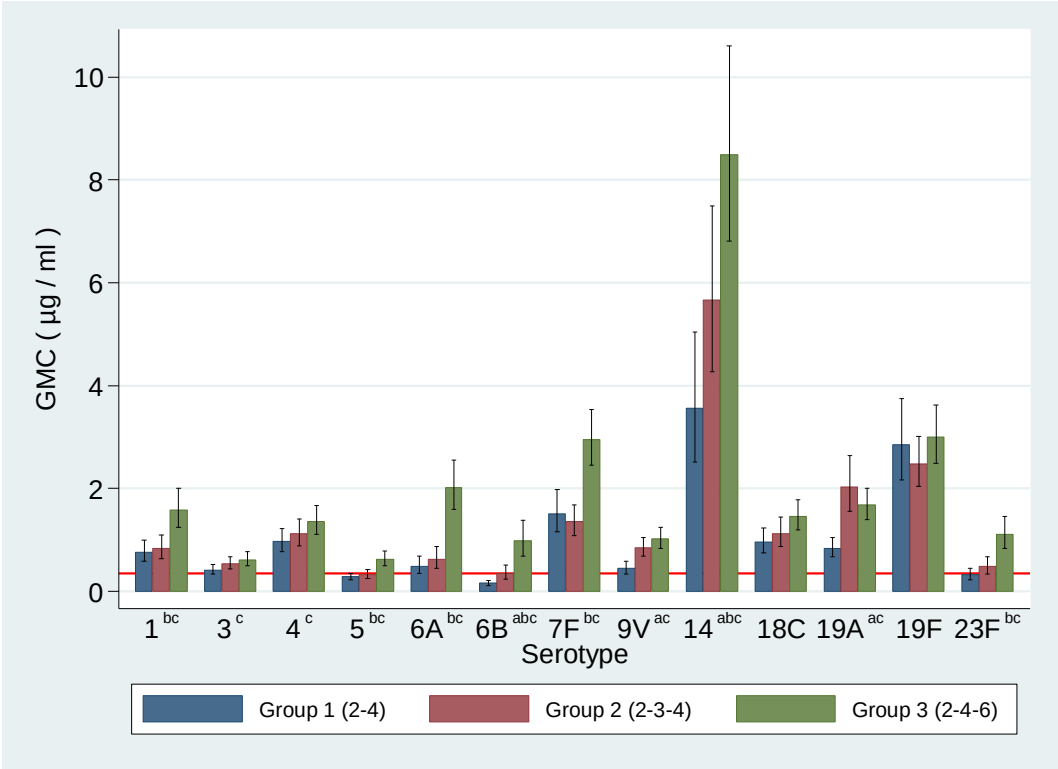

Figure 2: Pneumococcal IgG GMCs following primary vaccination for each serotype and group. a b c: p<0.05 comparing groups 1 and 2, 2 and 3 and 1 and 3 respectively. Black capped lines indicate 95% confidence intervals, solid horizontal red line indicates 0.35µg/mL.

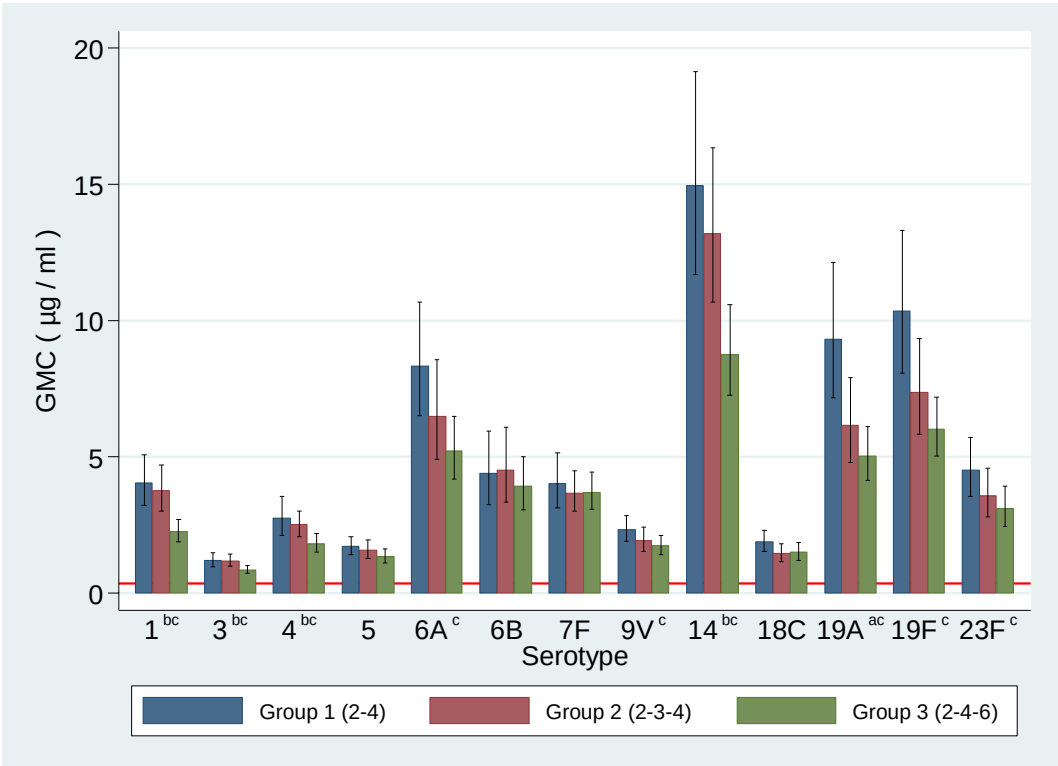

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Figure 3: Pneumococcal IgG GMCs following booster vaccination for each serotype and group. a b c: $p<0.05$ comparing groups 1 and 2, 2 and 3 and 1 and 3 respectively. Black capped lines indicate 95% confidence intervals, solid horizontal red line indicates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

Figures

	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)
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 V1 (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)
Maternal pertussis vaccine	12 (18)	8 (12)	12 (17)

Table 1: Baseline characteristics by group. Median (range) or n (%).

	Baseline	Post primary immunisations		
	All	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)
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 2: Proportion of infants with protective antibody concentrations (IgG≥0.35µg/mL) at baseline and 1 month after final primary vaccination. Proportion (95% CI). a b c: p<0.05 comparing groups 1 and 2, 2 and 3 and 1 and 3 respectively; *p<0.001

Serotype	Pre-booster vaccination			Post booster vaccination		
	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)
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)

Table 3: Proportion of infants with protective antibody concentrations (IgG≥0.35µg/mL) at prior to booster vaccination (12 months) and 1 month after booster vaccination. Proportion (95% CI). a b c: p<0.05 comparing groups 1 and 2, 2 and 3 and 1 and 3 respectively; *p<0.001

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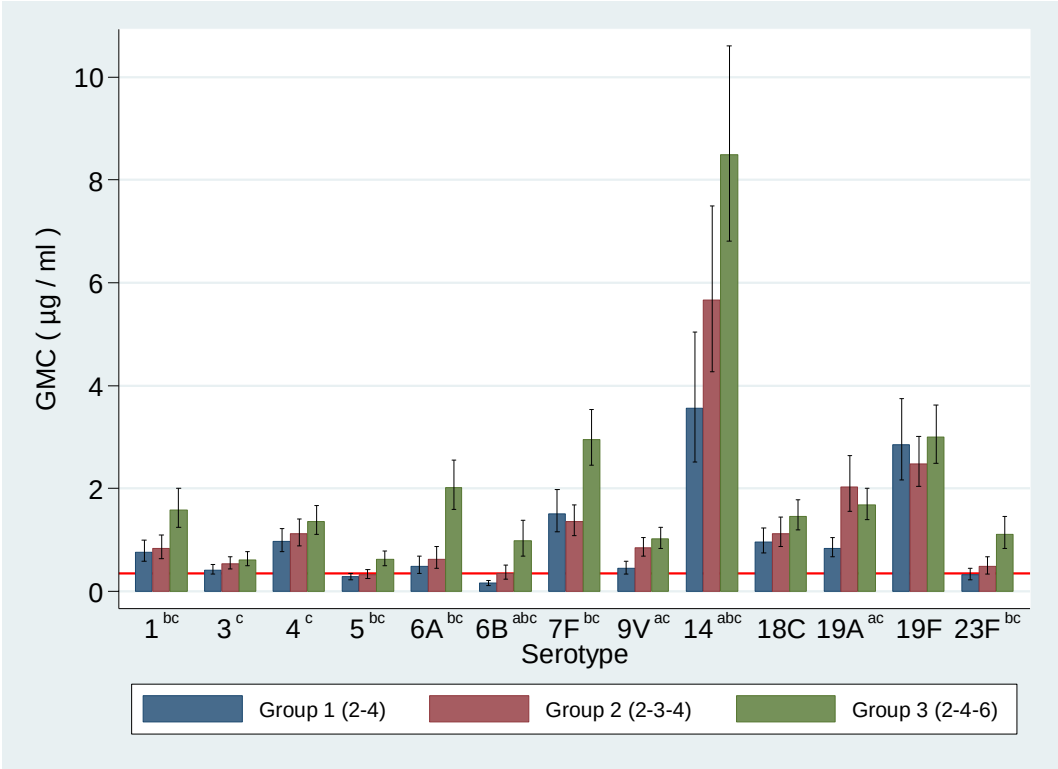
Figures

Primary schedule had significant impact on vaccine immunogenicity for all but 2 serotypes (ST 18C and 19F). The extended schedule resulted in higher IgG GMCs compared to the reduced dose schedule (11 serotypes) and accelerated schedule (7 serotypes). The accelerated schedule was superior to the reduced dose schedule for 4 serotypes (

Figures



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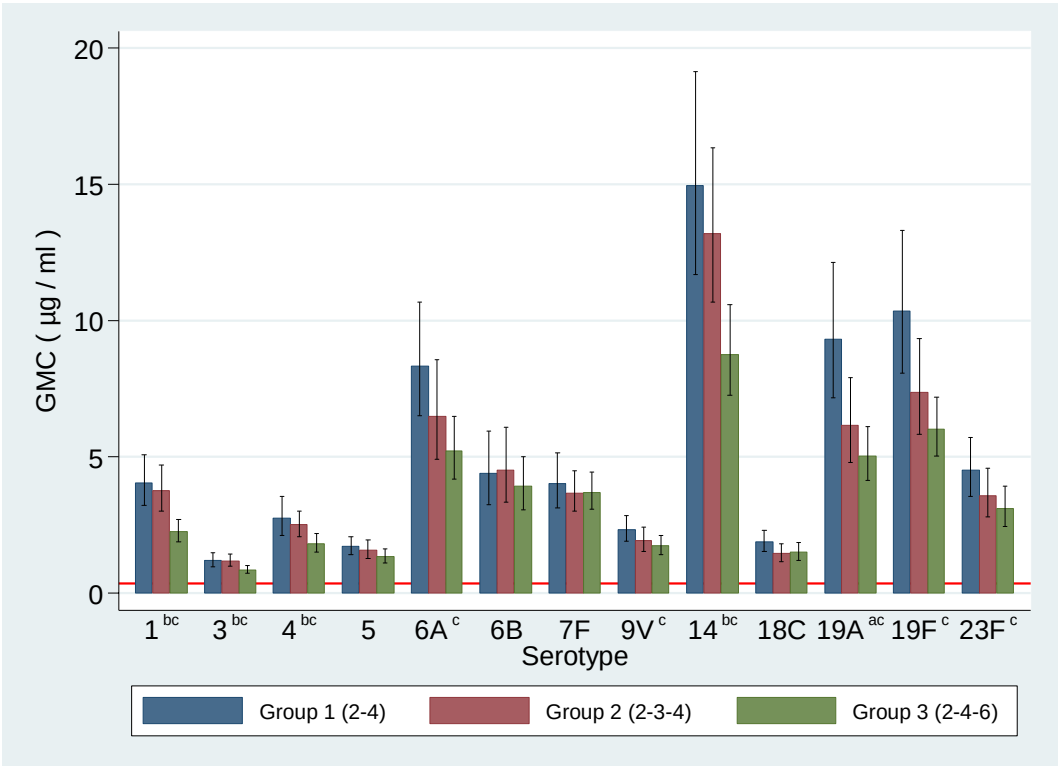

Figures

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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)

Table 3: Proportion of infants with protective antibody concentrations (IgG≥0.35µg/mL) at prior to booster vaccination (12 months) and 1 month after booster vaccination. Proportion (95% CI). a b c: p<0.05 comparing groups 1 and 2, 2 and 3 and 1 and 3 respectively; *p<0.001

and supplementary table 2).

Figures

Overall protection (defined as the number of serotypes with protective antibody concentrations per participant) was lower in the reduced dose group compared to the accelerated ($p=0.027$) or extended schedule ($p<0.001$) (supplementary figure 1). Seroprotection against less than 8 serotypes was seen in 30%, 17% and 4% of participants who received reduced dose, accelerated and extended schedules respectively (supplementary table 3).

Booster vaccination

At 12 months of age (6 - 8 months after the final PCV13 dose) waning of the pneumococcal antibody concentrations was evident with lower rates of seroprotection (Table 3 and supplementary table 4). Antibody concentrations remained higher in the extended schedule group compared to the reduced dose group (10 serotypes) or accelerated schedule group (11 serotypes), the accelerated schedule was superior to the reduced dose schedule for serotype 14 only.

Following booster vaccination mean fold increases in antibody concentrations varied between 3.5 (serotype 14, group 3) and 36.7 (serotype 23F, group 1). Similar to previous time points, marked variation in antibody concentrations between serotypes and groups was apparent (Figure 3). Despite these variations a high proportion of infants had protective concentrations (Table 3).

Contrary to findings following primary vaccination, the extended schedule group had lower GMCs compared to the reduce dose schedule (9 serotypes) and accelerated schedule (4 serotypes). The accelerated schedule was inferior to the reduced dose schedule for one serotype (19A) (supplementary table 5). These lower antibody concentrations were despite the higher GMCs seen in the extended schedule group prior to booster vaccination and are

due to lower fold increases in concentrations following booster vaccination in the extended schedule group (supplementary figure 2).

Predictors of antibody concentrations

Increased odds of seroprotection at 2 months of age were seen for 4 serotypes with each week increased gestation: ST 6A (OR 1.34, 95% CI 1.12-1.60; p=0.001), ST 14 (OR 1.25, 95% CI 1.12-1.41; p<0.001), ST 19A (OR 1.27, 95% CI 1.12-1.45; p<0.001) and ST 19F (OR 1.29, 95% CI 1.09-1.52; p=0.003). Later gestation was also associated with an increase in post-primary IgG concentrations for 3 serotypes: ST 1 (6% increase per week, 95% CI 0.9-12; p=0.021), ST 3 (8% increase per week, 95% CI 4-14, p<0.001) and ST 7F (8% increase per week, 95% CI 3-13; p=0.002).

Additionally, receipt of antenatal steroids was associated with decreased odds of seroprotection at baseline for 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) (multivariable logistic analysis adjusted for gestation) but not at any other time point.

Antibody concentrations after booster PCV13 dose were affected by priming schedule and pre-existing antibody levels only.

Safety and adverse events

There were no significant differences in the frequency and severity of local and systemic adverse events between vaccination schedules.

Figures

77 serious adverse events (SAEs) were reported (including 2 deaths). SAEs were predominantly acute respiratory infections including bronchiolitis. There were 3 suspected unexpected serious adverse reactions (SUSARs), 2 episodes of necrotising enterocolitis within a week of vaccination (1 requiring surgical intervention) and 1 participant had post-vaccination desaturations and bradycardias requiring readmission; all 3 infants made a good recovery.

Fever ≥38°C was reported after 4.7% of primary PCV13 vaccinations and pain after 27.1% (severe 1.0%). Injection site erythema and swelling were infrequent. Following the booster dose fever was reported in 24% of participants and injection site pain, erythema and swelling was experienced by 32%, 10% and 6% of participants respectively (supplementary table 6).

Discussion

This is the first study of PCV13 schedules in premature infants and demonstrates their ability to generate seroprotective antibody concentrations for all vaccine serotypes following vaccination, particularly after the booster dose at 12 months of age. Priming schedule significantly affected the immunogenicity of both the primary and booster doses.

Serotype-specific immunogenicity varied with lowest IgG GMCs seen for serotypes 3, 5 and 6B after the primary course and serotypes 3, 9V and 18C after the booster dose, these findings consistent with term infants[9,21]. However, when compared to previous term (PCV13) and preterm (PCV7) studies, antibody concentrations after primary and booster vaccination are lower, resulting in reduced seroprotection following primary vaccination[6,9,10,22].

Comparing schedules, the most prominent finding was the contrasting immunogenicity at different time points, with the reduced dose schedule generating inferior antibody

concentrations after the primary course but the better response to the 12 month dose. The increased post-primary IgG GMCs following 3 doses is consistent with two meta-analysis of primary schedules in term infants and, whilst they did not find any benefit of an older age of final vaccination, delaying the final dose in premature infants to allow for maturation of the immune system is likely to result in the higher antibody concentrations seen in those receiving the extended schedule[23–26].

The variation in booster dose immunogenicity was unexpected as priming schedule has not consistently shown an effect on the generation of immunological memory and PCV booster vaccine responses in term infants[23,27]. Infants receiving the reduced dose schedule had higher antibody concentrations than those who had 3 priming doses. The improved post-booster immunogenicity of few priming doses is well described for meningococcal conjugate vaccination and thought to be due to lower antigen concentrations favouring differentiation of B lymphoblasts to memory B cells instead of antibody-generating plasma cells[13,14]. Reports in PCV are rare; a study of Fijian infants receiving 1, 2 or 3 doses of PCV7 prior to PPV at 12 months of age found that infants who only had one priming dose had higher GMCs following PPV for serotypes 4, 9V, 19F and that there was no significant differences between 2 and 3 priming doses[12]. Similarly, infants receiving a lower antigen-containing investigational tetravalent PCV had higher booster responses than those who had received the preparation containing more antigen[28]. Thus, within our cohort, improved memory formation in the reduced schedule group may have contributed to their higher antibody concentrations at 13 months.

Infants who had received the extended schedule had lower fold increase in antibody concentrations following booster vaccination than those receiving either the reduced dose or

accelerated schedule. The negative impact of pre-existing antibody on vaccine response has been demonstrated following booster doses of other vaccines and may be due to formation of immune complexes decreasing the availability of the vaccine antigen and / or B cell receptor mediated negative feedback mechanisms analogous with those described for the effect of high maternal antibody concentrations on primary vaccine responses[29–33]. Accordingly, the higher antibody concentrations at 12 months seen in the extended schedule infants may have interfered with their response to the booster dose.

Within our premature cohort, increased birth gestation was associated with increased immunogenicity. This has previously been described for other vaccines but not PCV13 and reflects deficiencies in both the innate and adaptive immune systems in these more premature infants including a reduced pro-inflammatory cytokine response and decreased circulating B cells[34–39].

Limitations

The study had some limitations. The different ages of infants at blood sampling after primary vaccination between the groups must be considered; the antibody concentrations at 7 months for babies in the reduced dose and accelerated groups are not known. It is possible, but unlikely given the low rates of carriage of vaccine-serotype pneumococci, that infants in those groups may have seen a rise in their antibody concentrations between their 5 month sample and 7 months of age due to natural exposure[40]. 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].

As the objectives of this study were to look at schedule differences within the premature population we did not include a term comparator group which could have quantified any differences with the general population.

Figures

Additionally, we did not include any assessment of functional activity of the antibodies detected. Opsonophagocytic antibody (OPA) titres may have allowed us to assess the clinical impact of the between schedule differences in more detail. However, a meta-analysis of primary PCV schedules in term infants showed a good relationship between ELISA measured IgG concentration and opsonophagocytic antibody titres[24].

Conclusion

PCV13 is well tolerated in premature infants, however immunogenicity is related to priming schedule and different schedules offer increased protection at different ages. Implemented schedules most therefore reflect the local epidemiology of IPD as the optimal schedule for preterm infants depends on when they are most at risk of invasive disease. The duration of protection remains unknown.

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Figures

Figures

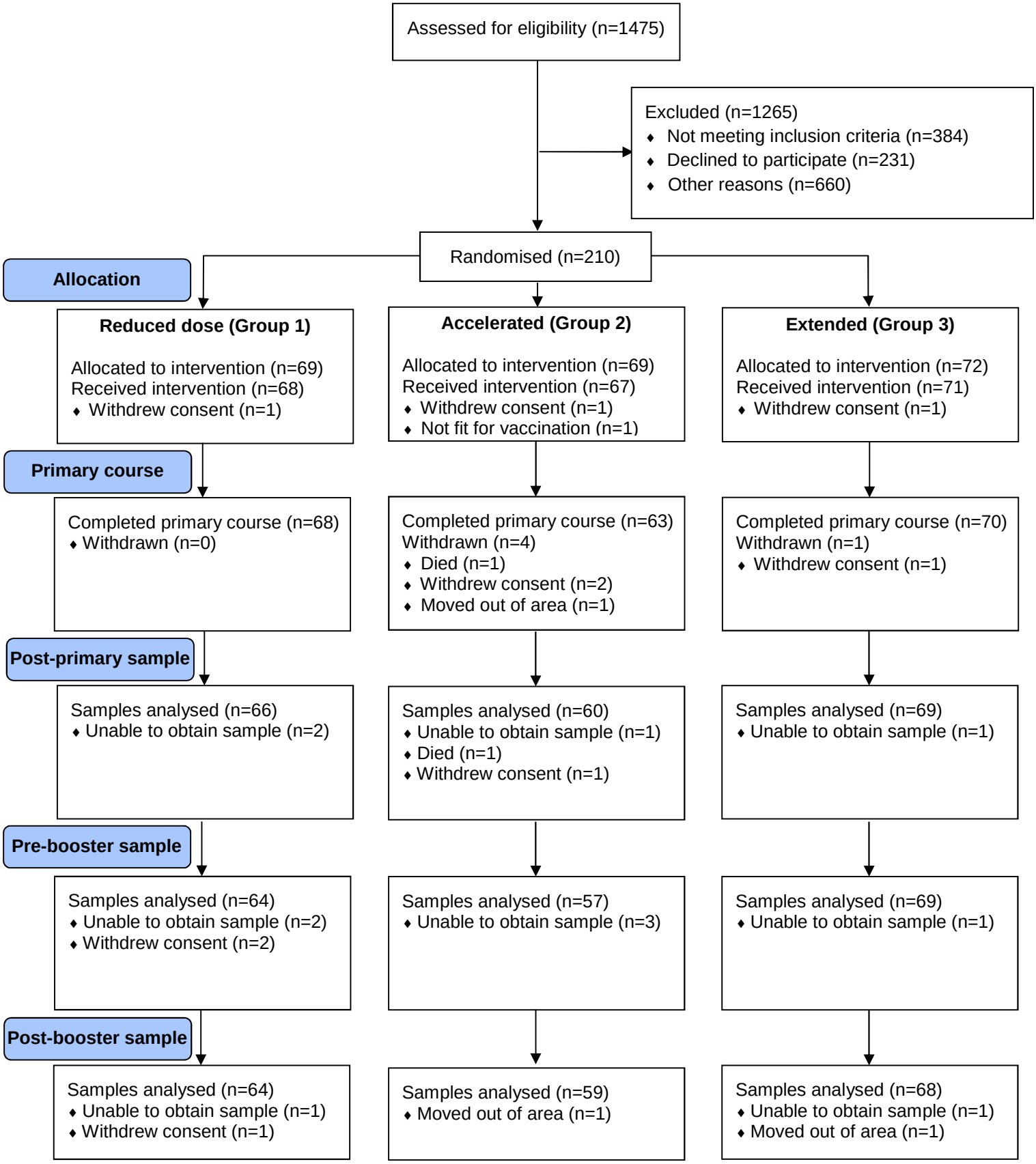


Figure 1: Consort diagram

Figures

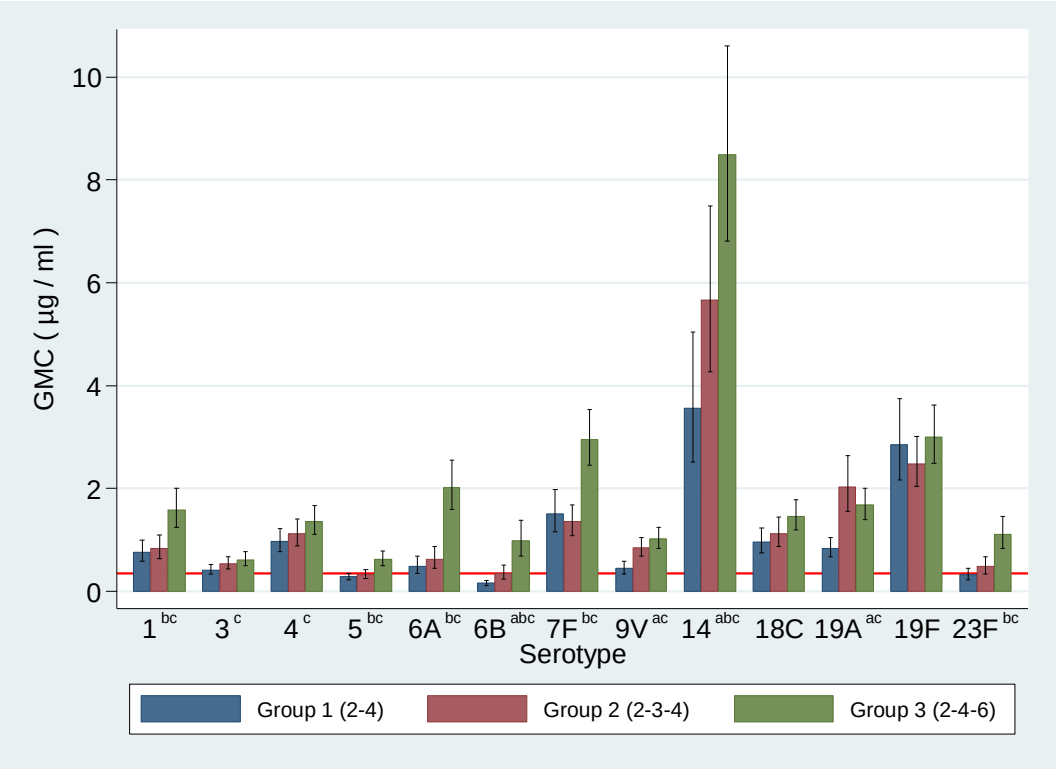


Figure 2: Pneumococcal IgG GMCs following primary vaccination for each serotype and group. a b c: p<0.05 comparing groups 1 and 2, 2 and 3 and 1 and 3 respectively. Black capped lines indicate 95% confidence intervals, solid horizontal red line indicates 0.35µg/mL.

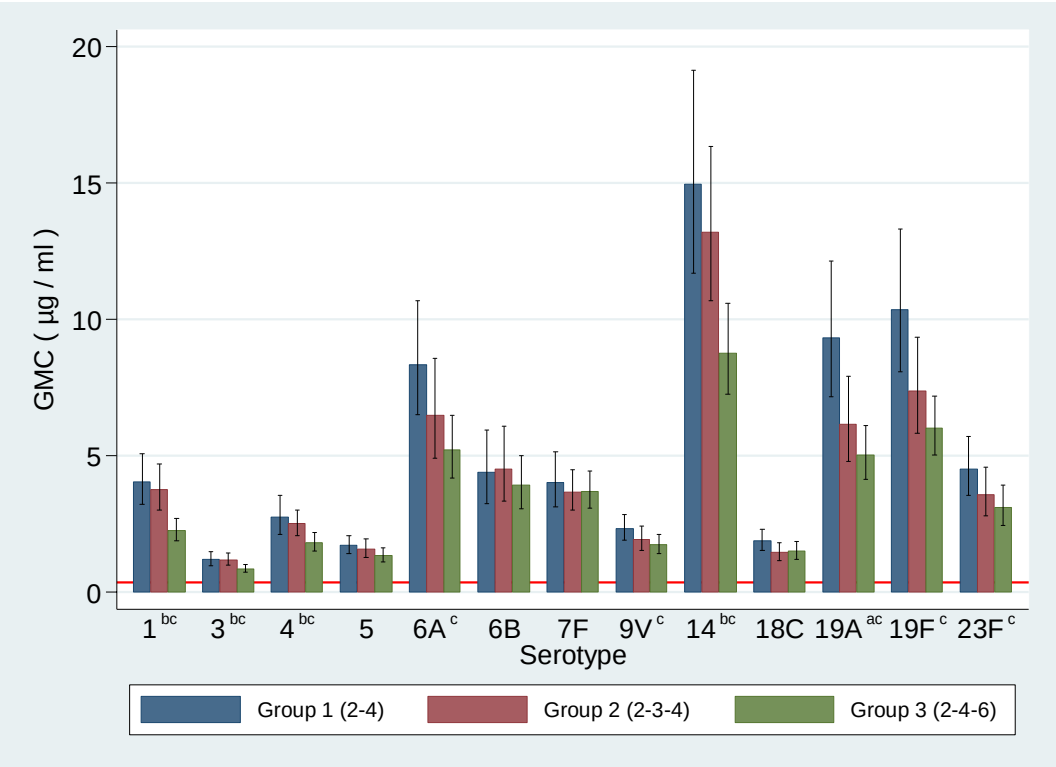


Figure 3: Pneumococcal IgG GMCs following booster vaccination for each serotype and group. a b c: p<0.05 comparing groups 1 and 2, 2 and 3 and 1 and 3 respectively. Black capped lines indicate 95% confidence intervals, solid horizontal red line indicates 0.35µg/mL.

Figures

Tables

	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)
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 V1 (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)
Maternal pertussis vaccine	12 (18)	8 (12)	12 (17)

Table 1: Baseline characteristics by group. Median (range) or n (%).

	Baseline	Post primary immunisations		
	All	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)
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 2: Proportion of infants with protective antibody concentrations (IgG≥0.35µg/mL) at baseline and 1 month after final primary vaccination. Proportion (95% CI). a b c: p<0.05 comparing groups 1 and 2, 2 and 3 and 1 and 3 respectively; *p<0.001

Serotype	Pre-booster vaccination			Post booster vaccination		
	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)
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)

Table 3: Proportion of infants with protective antibody concentrations (IgG≥0.35µg/mL) at prior to booster vaccination (12 months) and 1 month after booster vaccination. Proportion (95% CI). a b c: p<0.05 comparing groups 1 and 2, 2 and 3 and 1 and 3 respectively; *p<0.001

Supplementary tables

Serotype	Number > LLQ (%)	IgG GMC (95% CI)		
		Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)
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)

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

Serotype	IgG GMC (95% CI)		
	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)
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*}

2. IgG GMCs following primary immunisation course for each serotype and group.
***a b c*: p<0.05 comparing groups 1 and 2, 2 and 3 and 1 and 3 respectively; *p<0.001**

Number of ST IgG>0.35µg/mL	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)
0	1 (2)	0	0
1	0	1 (2)	0
2	0	0	0
3	2 (3)	1 (2)	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
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)

3. Number of serotypes with protective concentrations per participants for each. n (%)

Serotype	IgG GMC (95% CI)		
	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)
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*}

Supplementary tables

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*]

4. IgG GMCs prior to booster vaccination (12 months old) for each serotype and group.
***a b c*: p<0.05 comparing groups 1 and 2, 2 and 3 and 1 and 3 respectively; *p<0.001**

Serotype	IgG GMC (95% CI)		
	Reduced dose (Group 1)	Accelerated (Group 2)	Extended (Group 3)
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]

5. 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; *p<0.001**

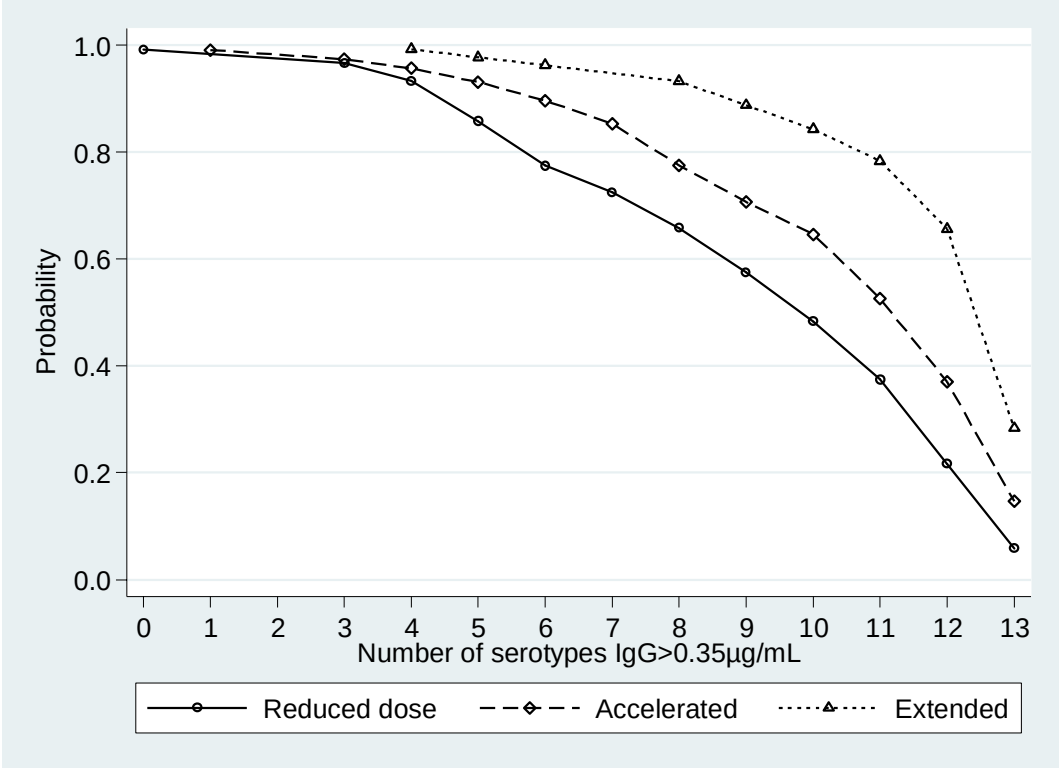
Visit	Reaction	Group	N	Any		Severe*	
				n	Proportion (CI)	n	Proportion (CI)
1	Fever	1	67	3	0.05 (0.01-0.13)	0	0.00 (0.00-0.05)
		2	63	1	0.02 (0.00-0.09)	1	0.02 (0.00-0.09)
		3	65	2	0.03 (0.00-0.11)	0	0.00 (0.00-0.06)
	Erythema	1	67	0	0.00 (0.00-0.05)	0	0.00 (0.00-0.05)
		2	63	1	0.02 (0.00-0.09)	0	0.00 (0.00-0.06)
		3	65	3	0.05 (0.01-0.13)	1	0.02 (0.00-0.08)
	Swelling	1	67	3	0.05 (0.01-0.13)	0	0.00 (0.00-0.05)
		2	63	2	0.03 (0.00-0.11)	0	0.00 (0.00-0.06)
		3	65	4	0.06 (0.02-0.15)	1	0.02 (0.00-0.08)
	Tenderness	1	67	15	0.22 (0.13-0.34)	2	0.03 (0.00-0.10)
		2	63	21	0.33 (0.22-0.46)	1	0.02 (0.00-0.09)
		3	65	19	0.29 (0.19-0.42)	0	0.00 (0.00-0.06)
2	Fever	2	59	3	0.05 (0.01-0.14)	0	0.00 (0.00-0.06)
	Erythema	2	59	1	0.02 (0.00-0.09)	0	0.00 (0.00-0.06)
	Swelling	2	59	1	0.02 (0.00-0.09)	0	0.00 (0.00-0.06)
	Tenderness	2	59	17	0.29 (0.18-0.42)	2	0.03 (0.00-0.12)
3	Fever	1	67	3	0.05 (0.01-0.13)	0	0.00 (0.00-0.05)
		2	55	3	0.06 (0.01-0.15)	2	0.04 (0.00-0.13)
		3	62	5	0.08 (0.03-0.18)	0	0.00 (0.00-0.06)
	Erythema	1	67	0	0.00 (0.00-0.05)	0	0.00 (0.00-0.05)
		2	55	2	0.04 (0.00-0.13)	0	0.00 (0.00-0.07)
		3	62	1	0.02 (0.00-0.09)	0	0.00 (0.00-0.06)
	Swelling	1	67	2	0.03 (0.00-0.10)	0	0.00 (0.00-0.05)
		2	55	1	0.02 (0.00-0.10)	0	0.00 (0.00-0.07)
		3	62	2	0.03 (0.00-0.11)	0	0.00 (0.00-0.06)
	Tenderness	1	67	22	0.33 (0.22-0.45)	0	0.00 (0.00-0.05)
		2	55	14	0.26 (0.15-0.39)	0	0.00 (0.00-0.07)
		3	62	16	0.26 (0.16-0.39)	0	0.00 (0.00-0.06)
5	Fever	3	67	4	0.06 (0.02-0.15)	2	0.03 (0.00-0.11)
	Erythema	3	67	4	0.06 (0.02-0.15)	2	0.03 (0.00-0.10)
	Swelling	3	67	4	0.06 (0.02-0.15)	0	0.00 (0.00-0.05)
	Tenderness	3	67	13	0.19 (0.11-0.31)	0	0.00 (0.00-0.05)
7	Fever	1	57	16	0.28 (0.17-0.42)	7	0.12 (0.05-0.24)
		2	50	14	0.28 (0.16-0.43)	4	0.08 (0.02-0.19)
		3	63	11	0.18 (0.09-0.29)	6	0.10 (0.04-0.20)
	Erythema	1	57	6	0.11 (0.04-0.22)	3	0.05 (0.01-0.15)
		2	50	5	0.10 (0.03-0.22)	4	0.08 (0.02-0.19)

Supplementary tables

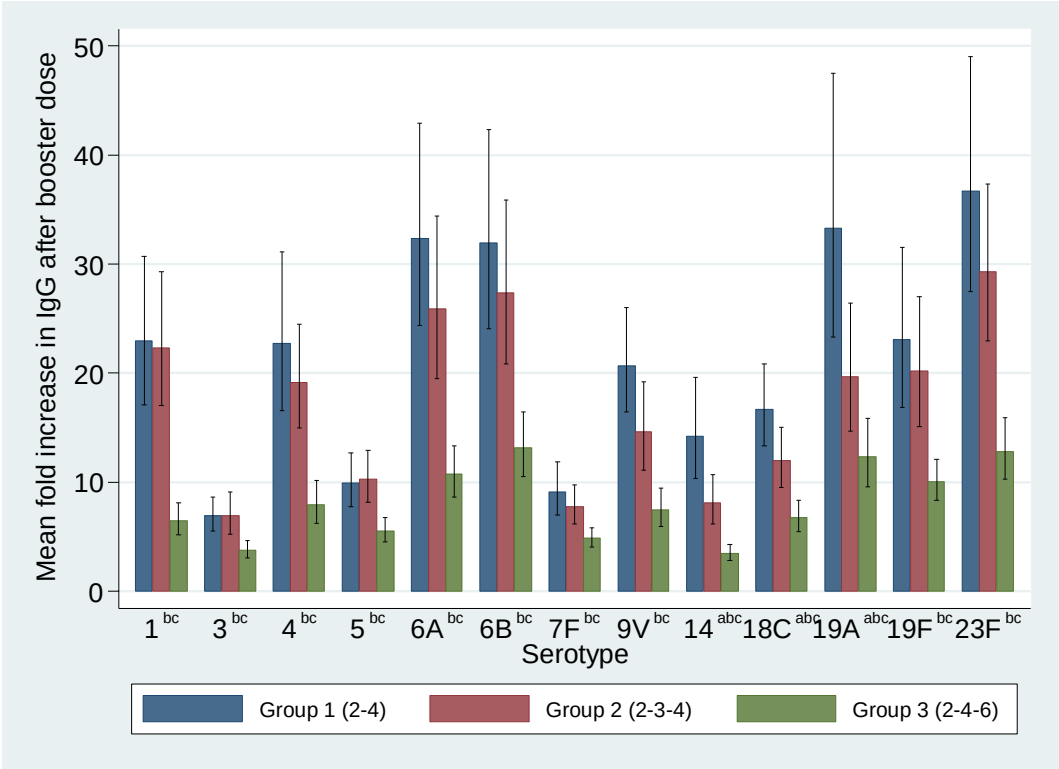
		3	63	5	0.08 (0.03-0.18)	1	0.02 (0.00-0.09)
	Swelling	1	57	4	0.07 (0.02-0.17)	2	0.04 (0.00-0.12)
		2	50	3	0.06 (0.01-0.17)	3	0.06 (0.01-0.17)
		3	63	3	0.05 (0.01-0.13)	1	0.02 (0.00-0.09)
	Tenderness	1	57	18	0.32 (0.20-0.45)	2	0.04 (0.00-0.12)
		2	50	18	0.36 (0.23-0.51)	1	0.02 (0.00-0.11)
		3	63	18	0.29 (0.18-0.41)	0	0.00 (0.00-0.06)

6. PCV13 injection site adverse events and fever following vaccination. Any: ≥15mm erythema or swelling, fever≥38°C. Severe: ≥30mm erythema or swelling, fever≥39°C

Supplementary figures



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



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 groups 1 and 2, 2 and 3 and 1 and 3 respectively.