Original Investigation

Immunogenicity of 13-Valent Pneumococcal Conjugate Vaccine Administered According to 4 Different Primary Immunization Schedules in Infants

A Randomized Clinical Trial

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IMPORTANCE Immunization schedules with pneumococcal conjugate vaccine (PCV) differ among countries regarding the number of doses, age at vaccinations, and interval between doses.

OBJECTIVE To assess the optimal primary vaccination schedule by comparing immunogenicity of 13-valent PCV (PCV13) in 4 different immunization schedules.

DESIGN, SETTING, AND PARTICIPANTS An open-label, parallel-group, randomized clinical trial of healthy term infants in a general community in the Netherlands conducted between June 30, 2010, and January 25, 2011, with 99% follow-up until age 12 months.

INTERVENTIONS Infants (N = 400) were randomly assigned (1:1:1:1) to receive PCV13 either at ages 2, 4, and 6 months (2-4-6); at ages 3 and 5 months (3-5); at ages 2, 3, and 4 months (2-3-4); or at ages 2 and 4 months (2-4), with a booster dose at age 11.5 months.

MAIN OUTCOMES AND MEASURES Primary outcome measure was antibody geometric mean concentrations (GMCs) against PCV13-included serotypes 1 month after the booster dose measured by multiplex immunoassay. Secondary outcomes included GMCs measured 1 month after the primary series, at 8 months of age, and before the booster.

RESULTS The primary outcome, GMCs at 1 month after the booster dose, was not significantly different between schedules for 70 of 78 comparisons. The 2-4-6 schedule was superior to the 2-3-4 schedule for serotypes 18C (10.2 μg/mL [95% CI, 8.2-12.7] vs 6.5 μg/mL [95% CI, 5.4-7.8]) and 23F (10.9 μg/mL [95% CI, 9.0-13.3] vs 7.3 μg/mL [95% CI, 5.8-9.2]) and superior to the 2-4 schedule for serotypes 6B (8.5 μg/mL [95% CI, 7.1-10.2] vs 5.1 μg/mL [95% CI 3.8-6.7]), 18C (6.6 μg/mL [95% CI, 5.7-7.7]), and 23F (7.2 μg/mL [95% CI, 5.9-8.8]). For serotype 1, the 3-5 schedule (11.7 μg/mL [95% CI, 9.6-14.3]) was superior to the other schedules. Geometric mean concentrations for all 13 serotypes ranged between 1.6 and 19.9 μg/mL. Secondary outcomes demonstrated differences 1 month after the primary series. The 2-4-6 schedule was superior compared with the 3-5, 2-3-4, and 2-4 schedules for 3, 9, and 11 serotypes, respectively. Differences between schedules persisted until the booster dose.

CONCLUSIONS AND RELEVANCE The use of 4 different PCV13 immunization schedules in healthy term infants resulted in no statistically significant differences in antibody levels after the booster dose for almost all serotypes. The choice of PCV schedule will require a balance between the need for early protection and maintaining protection between the primary series and the booster.

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The World Health Organization (WHO) estimated that more than 800 000 children younger than 5 years died from pneumococcal disease in 2000, making it the leading vaccine-preventable cause of death.[1] Since the licensure in 2000 of the first 7-valent pneumococcal polysaccharide conjugate vaccine (PCV) for infants, many countries have added PCV to their existing national immunization programs. As a result, PCV immunization schedules differ between countries with respect to number of doses, age at vaccinations, and intervals between doses.[2] Moreover, some countries implemented a catch-up program for older children. Despite these differences, the reported overall decline in invasive pneumococcal disease (IPD) in hospitalized children younger than 5 years several years after implementation is approximately 60% in Western countries.[3-6]

Serum antibody concentrations as measured with the standardized WHO enzyme-linked immunosorbent assay (ELISA) are used as correlates of protection against IPD in children.[7] Higher antibody levels may be achieved by delaying the first vaccination from 2 to 3 months of age because of maturation of the immune response in infants.[8] Previous immunogenicity studies also highlighted that longer intervals between doses yielded higher antibody levels.[9,10] Moreover, small differences favoring a full-dose schedule (3 doses + 1) vs a reduced-dose schedule (2 doses + 1) were observed for serotypes 6B and 23F.[11] The optimal vaccine schedule for infants should provide maximal, sustained direct and indirect protection against IPD while using a minimal number of doses. The latter is particularly relevant in the context of overcrowded national immunization programs, public resistance to vaccines, and cost-effectiveness estimates.[12] To assess the optimal priming regimen with respect to antibody induction, we compared immunogenicity of 4 different schedules using the 13-valent PCV (PCV13) (Prevnar 13; Pfizer).

DTaP-IPV-Hib *diphtheria, tetanus, acellular pertussis, inactivated poliovirus, and* Haemophilus influenzae *type b vaccine*

GMCs *geometric mean concentrations*

IPD *invasive pneumococcal disease*

MIA *multiplex immunoassay*

OPA *opsonophagocytosis assay*

PCV13 *13-valent pneumococcal conjugate vaccine*

Methods

Study Design

A single-center, phase 4, randomized controlled parallel-group trial was conducted in the Netherlands to investigate the effects of 4 different primary immunization schedules on the immune response against all serotypes included in PCV13 (ie, serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F). Between June 30, 2010, and January 25, 2011, 400 infants were randomly assigned (1:1:1:1) to receive PCV13 either at ages 2, 4, and 6 months (subsequently referred to as 2-4-6); at ages 3 and 5 months (3-5); at ages 2, 3, and 4 months (2-3-4); or at ages 2 and 4 months (2-4). All infants received a booster dose of PCV13 at age 11.5 months. Irrespective of the assigned schedule, diphtheria, tetanus, acellular pertussis, inactivated poliovirus, and *Haemophilus influenzae* type b vaccine (DTaP-IPV-Hib) (Pediacel; Sanofi Pasteur MSD) was administered to all infants at ages 2, 3, 4, and 11.5 months according to the Dutch national immunization program. According to predefined limits, the first dose at age 2 months was administered at 60 (±7) days, and the 1-month interval between subsequent vaccine doses was 28 (±7) days, whereas the 2-month interval was 56 (±7) days. Vaccines were administered intramuscularly in the right (PCV13) or left (DTaP-IPV-Hib) anterolateral thigh. Randomization was performed by a random number generator[13] using block randomization with randomly varying block size. Allocations were concealed in sequentially numbered envelopes that were opened during a home visit after enrollment of the infant. Study staff members and parents were aware of the child's allocated immunization schedule, but laboratory staff was not.

Participants

Parents of newborns eligible for routine childhood immunization were invited to participate, excluding those targeted for hepatitis B vaccination according to Dutch guidelines to prevent inclusion of children receiving different concomitant vaccines.[14] Healthy infants born after a gestation period of at least 37 weeks, not yet having received any infant vaccination, and living in the study area in the northwestern part of the Netherlands were eligible. Exclusion criteria were any known primary or secondary immunodeficiency, bleeding disorders, receipt of immunoglobulin or blood products, or a language barrier. At each visit, a brief survey was obtained from parents for baseline demographics, occurrence of illnesses, and medication usage, including prophylactic acetaminophen administration at the time of vaccination as this may significantly reduce antibody responses.[15] As PCV7 was introduced in the Dutch national immunization program for all infants born after March 31, 2006, in a 3 + 1 schedule, an additional fourth PCV13 vaccination was offered free of charge to all infants who received a 2 + 1 schedule in the current study at age 24 months. Participants did not receive any financial compensation. An independent national ethics committee (Centrale Commissie Mensgebonden Onderzoek[16]) approved the study protocol. Written informed consent was obtained from each infant's parent or guardian before enrollment. The study was performed in accordance with European guidelines for Good Clinical Practice, which includes provisions of the Declaration of Helsinki.

Serological Analyses

Venous blood samples of 2 mL were collected from all participants at 4 different time points: 1 month (28 days ±7 days) after the primary series (ie, postprimary), at 8 months of age (−7/+14 days), before the booster dose at age 11.5 months (±15 days), and 1 month (28 days ±7 days) after the booster dose. Blood sampling was postponed in case of fever (>38.5°C) within 24 hours before the planned visit to exclude possible interference of immune responses. Sera were stored at −80°C until analysis in the RIVM (Nether-

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lands National Institute for Public Health and the Environment, Bilthoven, the Netherlands). Pneumococcal serotype-specific IgG antibodies to the 13 pneumococcal serotypes were measured using a fluorescent bead-based multiplex immunoassay (MIA) as described previously.[17] Postprimary and postbooster antibody avidity indices were determined with MIA for all 13 serotypes using 0.5-M ammonium thiocyanate in a random subset of 40 samples per schedule with an IgG concentration of 0.25 μg/mL or greater for serotype 14.[18] Postprimary opsonophagocytotic activity was determined for serotypes 6B, 14, 19A, and 23F by multiplex opsonophagocytosis assay (OPA) in the same random subset of samples measured at University College London, Institute of Child Health, United Kingdom.[19] Prebooster and postbooster responses to routine DTaP-IPV-Hib antigens were determined according to availability of serum by MIA (for diphtheria, tetanus, pertussis, and *Haemophilus influenzae* type b) and a microneutralization test (for poliovirus) adopted from WHO guidelines.[20,21]

Primary and Secondary Outcomes

The primary objective was to determine superiority of one immunization schedule over another with regard to differences in serotype-specific geometric mean concentrations (GMCs) measured 1 month after the booster dose, at age 12 months. Main secondary objectives were (1) to assess differences in IgG GMCs between schedules measured at postprimary, 8 months, and prebooster time points; (2) to assess the functional activity (avidity, OPA) of the pneumococcal antibodies after the primary series and booster dose; (3) to determine antibody decline between the priming and booster vaccinations; and (4) to assess the immune responses to DTaP-IPV-Hib antigens before and after the booster dose.

Statistical Analysis

Taking into account correction for 6 mutual comparisons between the 4 vaccination schedules, a sample size of 80 evaluable participants was needed to enable detection of a 2-fold difference in GMCs of IgG antibodies directed against polysaccharide 6B, the serotype with the highest variance in antibody levels in different schedules based on previous data,[22] with 80% power and a significance level of .008 (ie, .05/6). Estimating that in up to 20% of infants less than the required serum would be available for testing, we aimed to include 100 participants per group.

Statistical superiority analysis was performed on the intention-to-treat population. For each randomization group, pneumococcal serotype-specific GMC/geometric mean titers (GMTs) and their 2-sided 95% confidence intervals were calculated. As exploratory analyses, percentages of vaccine recipients reaching an IgG threshold of 0.35 μg/mL for all time points and 1.0 μg/mL measured after the booster dose and reaching an OPA threshold of 1:8 were calculated. The avidity index was expressed as the mean percentage of antibodies that remained bound to the antigen. Antibody responses were also calculated for DTaP-IPV-Hib antigens and standard correlates of protection were used to interpret responses for each randomization group. Differences in antibody responses between groups were analyzed using analyses of variance and *P* values were adjusted for 6 multiple comparisons using Bonferroni correction. All data were analyzed using SPSS version 20.0 (IBM SPSS Statistics), all tests were 2-sided, and a *P* value of less than .05 was considered statistically significant.

Results

In total, 400 infants were enrolled, of whom 396 (99%) completed the study. In 1440 of 1590 attempts (90%), a sufficient blood sample was obtained and results were included in the intention-to-treat analyses. The missing samples were equally distributed over the 4 groups, and no child missed more than 1 sampling moment. Of the 1440 samples, 1427 (99%) were derived from participants with no protocol deviations (**Figure 1**). The baseline characteristics and age at visits between groups were similar (**Table**). Acetaminophen was administered prophylactically in less than 2% of the infants, independent of intervention group.

Primary Outcome Measure

One month after the booster dose, there were no differences in IgG GMCs between the schedules except for 8 of 78 comparisons (**Figure 2**, **Figure 3**, and eTable 1A in the Supplement). The 2-4-6 schedule was superior to the 2-3-4 schedule for serotypes 18C (10.2 μg/mL [95% CI, 8.2-12.7] vs 6.5 μg/mL [95% CI, 5.4-7.8]) and 23F (10.9 μg/mL [95% CI, 9.0-13.3] vs 7.3 μg/mL [95% CI, 5.8-9.2]) and superior to the 2-4 schedule for serotypes 6B (8.5 μg/mL [95% CI, 7.1-10.2] vs 5.1 μg/mL [95% CI, 3.8-6.7]), 18C (6.6 μg/mL [95% CI, 5.7-7.7]), and 23F (7.2 μg/mL [95% CI, 5.9-8.8]). For serotype 1, the 3-5 schedule (11.7 μg/mL [95% CI, 9.6-14.3]) was superior to the 2-4-6 (6.0 μg/mL [95% CI, 4.7-7.5]), 2-3-4 (6.2 μg/mL [95% CI, 5.0-7.6]), and 2-4 schedules (7.8 μg/mL [95% CI, 6.6-9.3]). Geometric mean concentrations for the 13 serotypes ranged between 1.6 μg/mL (95% CI, 1.3-1.9) for serotype 3 in the 2-4-6 schedule and 19.9 μg/mL (95% CI, 16.7-23.7) for serotype 6A in the 2-4 schedule.

Secondary Outcome Measures

Differences in IgG Levels After the Primary Series

One month after the primary series, significant differences in IgG levels existed between schedules for all serotypes (Figure 2, Figure 3, and eTable 1B in the Supplement) with GMCs for the 13 serotypes ranging between 0.09 μg/mL (95% CI, 0.07-0.11) for serotype 6B in the 2-4 schedule and 7.8 μg/mL (95% CI, 6.6-9.4) for serotype 6A in the 2-4-6 schedule. The 2-4-6 schedule was superior to the 3-5, 2-3-4, and 2-4 schedules for 3, 9, and 11 serotypes, respectively, but inferior to the 3-5 schedule for 1 serotype. The 3-5 schedule was superior to the 2-3-4 and 2-4 schedules for 5 and 11 serotypes, respectively. In addition, the 2-3-4 schedule was superior to the 2-4 schedule for 5 serotypes but inferior for 1 serotype. Differences in IgG levels between immunization schedules persisted at age 8 months and

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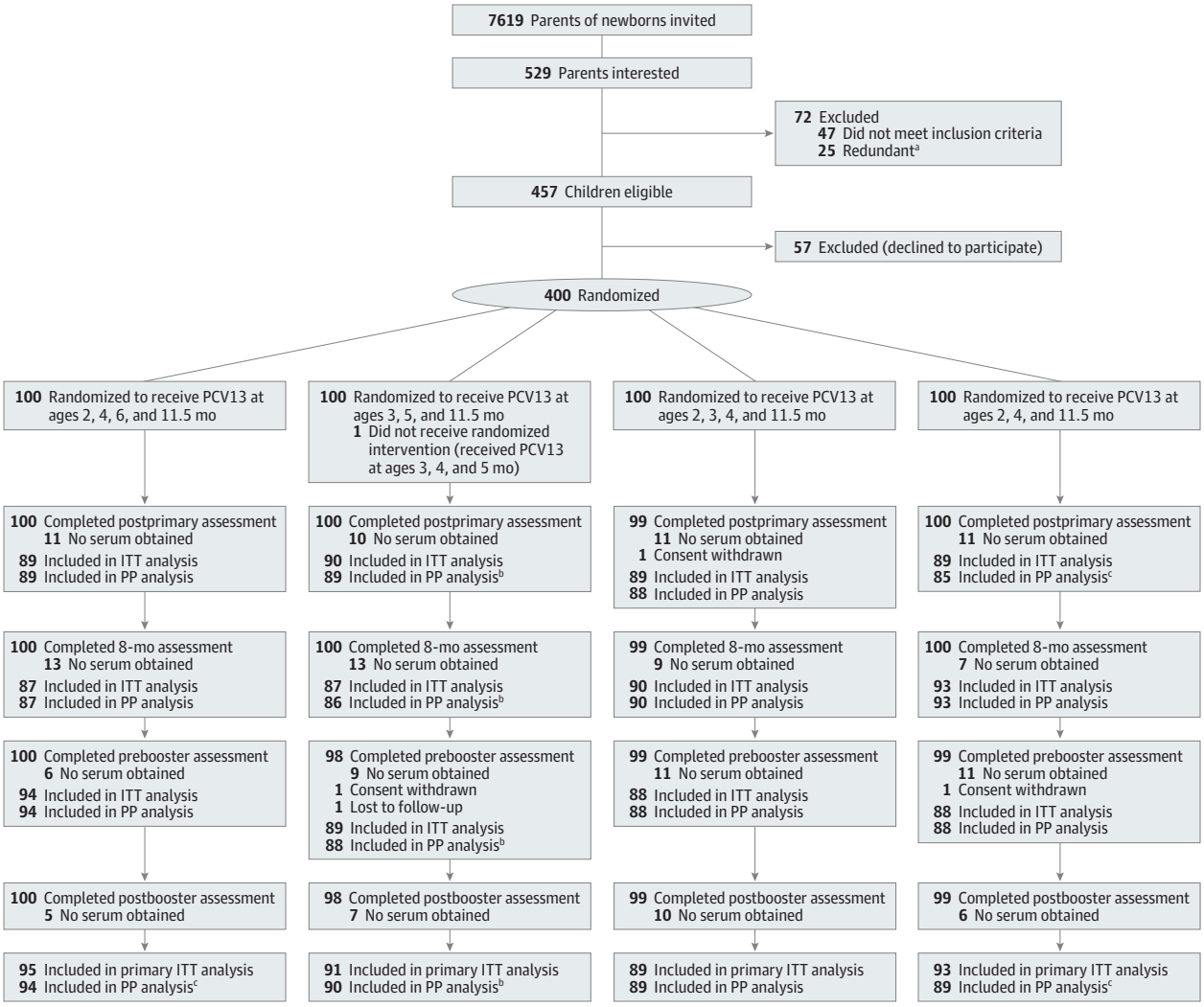
Figure 1. Enrollment and Study Participation



PCV13 indicates 13-valent pneumococcal conjugate vaccine; ITT, intention to treat; PP, per-protocol.

[a]Parents of children interested in participating in the study were considered redundant because the enrollment target had already been achieved and informed consent procedure was cancelled.

[b]Sample was excluded from PP analysis due to nonadherence with vaccination schedule.

[c]Samples were excluded from PP analysis due to nonadherence with blood sampling schedule.

before the booster dose at 11.5 months, except for the 2-3-4 vs 2-4 schedule (Figure 2 and eTable 1C-1D in the Supplement).

Differences in Functional Responses of Pneumococcal Antibodies

After the booster dose, except for a single comparison, there were no differences in avidity between schedules. After the primary series, higher avidity indices were observed after the primary time point for the 2-4-6 schedule, particularly when compared with the 2-3-4 schedule, which yielded the lowest avidity indices (eTable 2A-2B in the Supplement). The OPA GMTs demonstrated a similar pattern as observed for IgG levels, although the 2-3-4 schedule yielded higher titers for serotype 6B compared with the 3-5 schedule (eTable 2C in the Supplement). The correlation between log-transformed IgG levels and OPA values was between 0.5 and 0.8.

Pneumococcal Antibody Kinetics

Waning of antibody levels after the primary series was evident for nearly all serotypes, except for 6B in the 2-3-4, 2-4, and 3-5 schedules for which an increase in proportion of responders was observed in the prebooster period (eFigure 2 in the Supplement). High booster responses were observed for all serotypes, ranging from a 4-fold increase in GMCs for serotype 14 in the 2-4-6 schedule to a 22-fold increase for serotype 6B in the 2-4 schedule. Geometric mean concentrations after the booster dose exceeded

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Table. Characteristics of Participating Children

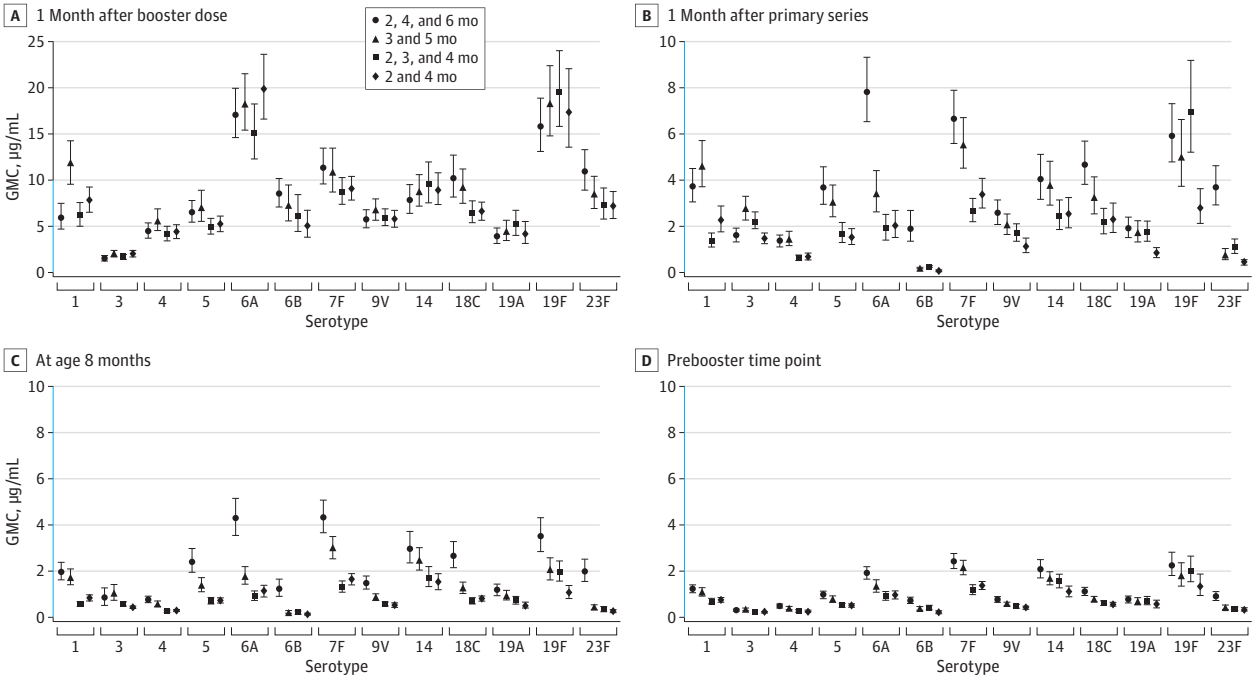
	Schedule[a]			
	2-4-6	3-5	2-3-4	2-4
Participants, No.	100	100	100	100
Male sex, No. (%)	57 (57.0)	53 (53.0)	55 (55.0)	49 (49.0)
Birth weight, mean (SD), g	3623 (450)	3584 (519)	3631 (458)	3531 (451)
Gestational age, mean (SD), wk	39.6 (1.2)	39.5 (1.2)	39.5 (1.2)	39.3 (1.2)
Presence of siblings <5 y, No. (%)[b]	47 (47.0)	53 (53.0)	43 (43.0)	48 (48.0)
Day care attendance, No. (%)[c]	61 (61.0)	59 (59.0)	49/99 (49.5)	48 (48.0)
Age at visit, mean (SD), mo				
2	1.9 (0.1)	1.9 (0.1)	1.9 (0.1)	1.9 (0.1)
3	2.9 (0.2)	2.9 (0.1)	2.9 (0.1)	2.9 (0.2)
4	3.9 (0.2)	3.9 (0.1)	3.9 (0.2)	3.9 (0.2)
5		4.9 (0.2)	4.9 (0.2)	4.9 (0.3)
6	5.9 (0.2)	5.9 (0.2)		
7	6.9 (0.2)			
8	8.1 (0.1)	8.1 (0.1)	8.1 (0.1)	8.1 (0.1)
11	11.5 (0.2)	11.5 (0.2)	11.5 (0.2)	11.5 (0.2)
12	12.5 (0.2)	12.4 (0.2)	12.5 (0.2)	12.5 (0.2)

[a] Infants were assigned to receive the 13-valent pneumococcal conjugate vaccine either at ages 2, 4, and 6 months (2-4-6); at ages 3 and 5 months (3-5); at ages 2, 3, and 4 months (2-3-4); or at ages 2 and 4 months (2-4).

[b] Information was asked at enrollment.

[c] Defined as at least 4 continuous hours per week with at least 1 child <5 years of age from a different family, asked at 4 months of age.

Figure 2. Pneumococcal Serotype-Specific Antibody GMCs Measured at 4 Different Time Points



Error bars indicate 95% CIs; GMCs, geometric mean concentrations.

postprimary levels, except for serotype 3 (Figure 2 and eTable 1A-D in the Supplement).

Immunogenicity of Coadministered DTaP-IPV-Hib Vaccine

Regarding coadministered DTaP-IPV-Hib vaccine, no significant differences between schedules in GMCs/GMTs were found, except for polio type 2 and diphtheria prebooster. One month after the booster dose, seroprotection/seropositivity for all DTaP-IPV-Hib components was high (90%-100%) in all groups (eTable 3A-3B in the Supplement).

Exploratory Outcome Measures

One month after the booster dose, nearly all participants reached the 0.35 µg/mL seroprotection threshold and 71% to 100% reached the 1.00-µg/mL threshold for all serotypes. One month after the primary series, seroprotection rates ranged be-

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tween 76% and 100% in all schedules but were lower for serotypes 6B (11%, 30%, and 40% for 2-4, 3-5, and 2-3-4, respectively) and 23F (58% for 2-4 and 68% for 3-5). The percentages of participants with OPA titer at or above the 1:8 threshold ranged between 69% and 100% (eTable 2C in the Supplement). Reverse cumulative distribution curves emphasize the influence of different cutoff values on the percentage of serotype-specific seroprotection for each of the vaccination schedules for postprimary time points (eFigure 1A-B in the Supplement).

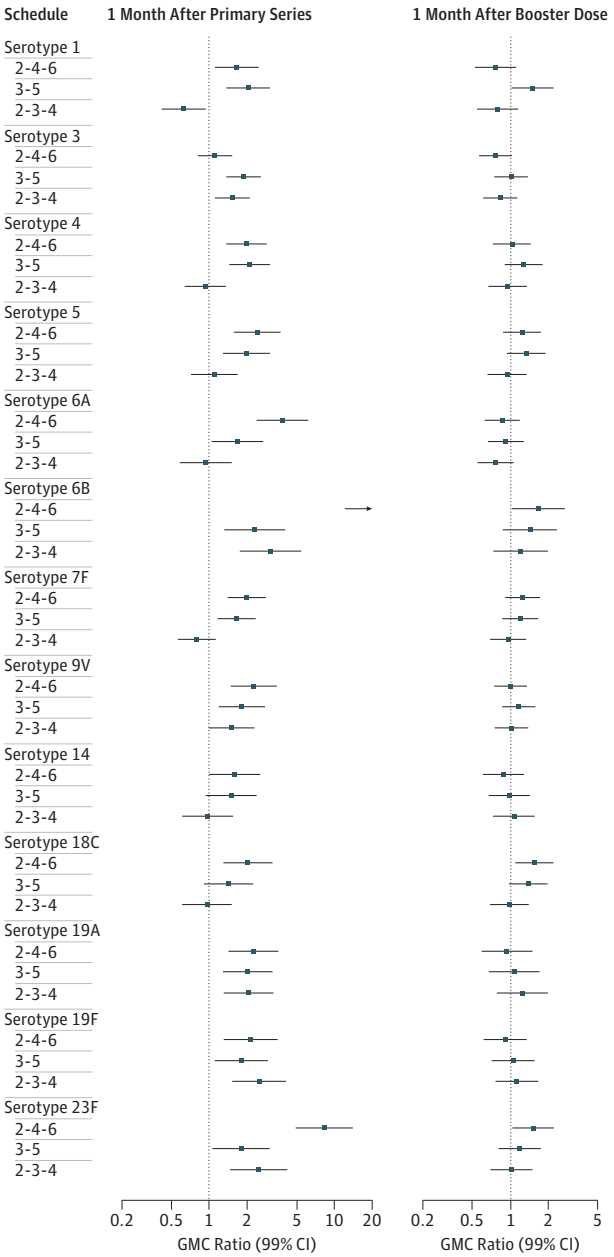
Discussion

To our knowledge, this is the first randomized controlled trial investigating immunogenicity of PCV13 in 4 different primary immunization schedules that are currently used in most high-income countries. The primary outcome of this study, GMCs 1 month after the booster dose, showed that there were no statistically significant differences between the 4 schedules in IgG levels for most serotypes. However, differences between schedules were noted in secondary analyses. After the primary series, the 2-4-6 schedule resulted in the highest IgG antibody levels, avidity, and opsonophagocytotic activity. The 3-5 schedule with only 2 primary doses was similar to the 2-4-6 schedule, with lower IgG levels only for serotypes 6A, 6B, and 23F. Both the 2-4-6 and 3-5 schedules were superior to the 2-3-4 and 2-4 schedules with respect to postprimary IgG levels for most serotypes. In an exploratory analysis, all children in all schedules reached equally high IgG levels above predefined seroprotection levels after the booster dose. Therefore, we do not expect a difference in clinical protection against IPD.

Our findings demonstrate that optimal timing of the primary series, ie, older age at vaccinations combined with longer intervals between vaccinations, is important to maintain optimal antibody levels during the period between the primary series and the booster dose. When PCV13 vaccination doses were delayed (3-5 vs 2-4), the antibody concentrations were significantly increased, reflecting the importance of immune maturation especially during the first months of life.[8] This is also illustrated by the fact that after the booster dose at age 11.5 months, differences between schedules largely disappeared. However, the effectiveness of the immune response has to be weighed against the need for early protection. To induce the earliest possible protection against pneumococcal disease in the absence of herd immunity, accelerated immunization schedules including neonatal vaccinations are preferred.[23]

Although the effects of age and vaccination interval cannot be completely separated in our study, schedules with a 2-month interval between doses compared with 1 month induced higher antibody concentrations in line with other studies investigating PCV[10] or diphtheria, tetanus, and pertussis vaccines.[9] Longer intervals also proved important for avidity maturation because the 2-3-4 schedule resulted in lower postprimary avidity indices compared with the 2-4-6, 3-5, and 2-4 schedules and differences between schedules disappeared with time, in line with a previous report.[23]

Figure 3. Pneumococcal Serotype-Specific Geometric Mean Concentration Ratios Between Schedules



The 4 dosing schedules were ages 2, 4, and 6 months (2-4-6); ages 3 and 5 months (3-5); ages 2, 3, and 4 months (2-3-4); and ages 2 and 4 months (2-4). The plots show the ratios of the 2-4-6, 3-5, and 2-3-4 schedules measured 1 month after the primary series and 1 month after the booster dose in the intention-to-treat population, with the 2-4 schedule as the reference. Not shown on the plot for the time point 1 month after the primary series is the GMC ratio for serotype 6B, which was 21.8 (99% CI, 12.4-38.3) for the 2-4-6 schedule compared with the 2-4 schedule. Error bars indicate 2-sided 99% CIs (adjusted for 6 multiple comparisons); GMCs, geometric mean concentrations.

The 3-dose 2-4-6 schedule resulted in the highest antibody levels after the primary series for most serotypes. However, the 2-dose 3-5 schedule proved to yield higher antibody concentrations for 5 serotypes than the other 3-dose schedule (2-3-4) and similar antibody concentrations for serotypes

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6B and 23F. This might suggest that the number of doses is less important than optimal timing of doses, although further study is required to corroborate this finding.

Long-term protection against IPD by PCV depends on a combination of persistence of protective serum antibody levels, immunological memory, and herd immunity.[24] In an exploratory analysis, we found that protective serum antibody levels defined as a level of greater than 0.35 μg/mL were high after the primary series in all schedules for most serotypes, except for 6B and 23F in the 2-4, 3-5, and 2-3-4 schedules. However, the use of this threshold value as seroprotective for all serotypes is likely to be inappropriate, being too high for serotype 6B and too low for others (eg, serotype 19F), and no threshold is defined for the 6 additional serotypes included in PCV13.[10,25] Interestingly, waning of antibody levels after the primary series was observed for all serotypes except for 6B, for which the antibody levels increased in the 2-3-4, 3-5, and 2-4 schedules. Because serotype 6B carriage in young children in the Netherlands was low during the study period, we think the increase in 6B antibodies reflects the poor immunogenic properties of the 6B antigen.[26] All schedules resulted in high booster responses and avidity maturation for nearly all serotypes, suggesting immunological memory. Herd immunity is established independent of the assigned schedule through reduction of carriage of vaccine serotypes, as previously demonstrated by nasopharyngeal carriage studies, even for 6B in the lowest immunogenic 2-4 schedule.[11,27]

In our study, we did not assess efficacy against clinical IPD, which would be the most clinically relevant outcome.[7] Efficacy studies are difficult to perform and costly because of sample size requirements and herd effects that may obscure vaccine schedule differences.[28] Head-to-head comparison of a 3 + 1 with a 2 + 1 schedule has been performed in a single cluster-randomized trial in Finland, enrolling infants up to 6 months of age with the 10-valent PCV (Synflorix; GlaxoSmithKline), although the overall number of IPD cases was small. No vaccine-type IPD occurred in the 3 + 1 schedule group, and 1 case of vaccine-type IPD (7F) occurred in the 2 + 1 schedule. This single case occurred within 2 weeks after the first vaccination, so it may not be a real vaccine failure.[29]

Strengths of our study include the longitudinal randomized controlled study design with an adequate sample size, performed in a single geographic region, with antibody levels measured at 4 different time points, small loss to follow-up, and a low rate of protocol deviations. In addition, prophylactic use of antipyretic drugs did not confound our results, because they were administered only incidentally to infants with an equal distribution over groups.[15]

To put the results of our study in perspective, some limitations have to be addressed. First, although good correlation has been demonstrated between MIA as used in this study and standard ELISA,[17] one needs to be cautious comparing the results with other studies. Because all samples were analyzed using validated MIA with independent duplicate runs performed in 1 laboratory using single bead batches, comparison of different schedules within this study is reliable. Second, the DTaP-IPV-Hib vaccine was administered to all infants at ages 2, 3, 4, and 11.5 months, irrespective of the assigned randomization group. Although no significant differences between schedules were found for DTaP-IPV-Hib components, we cannot exclude possible interference of the coadministrations on immune responses against PCV13. However, a previous study demonstrated that pneumococcal antibody responses after PCV7 vaccination were unaffected by either concurrent or sequential administration of DTaP-IPV-Hib and hepatitis B vaccines.[30] Third, our primary measure was IgG levels, and to corroborate our findings, functional assays were performed in a subset of samples and serotypes. Small discrepancies between the outcomes of the 3 assays might be explained by different subclasses of antibodies detected in these assays. The interpretation of differences in data generated with these assays in relation to protection remains to be further elucidated.[31]

Conclusions

The use of 4 different PCV13 immunization schedules in healthy term infants resulted in no statistically significant differences in antibody levels after the booster dose at 12 months of age for almost all serotypes. The choice of PCV schedule will require a balance between the need for early protection and maintaining protection between the primary series and the booster, in particular before herd effects offer clinical protection against vaccine serotype disease to as yet unvaccinated or incompletely vaccinated infants.[32] When herd immunity is established, clinical relevance of the observed differences in immune responses may become of minor importance.

ARTICLE INFORMATION

Author Contributions: Dr Spijkerman had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Schouls and Berbers contributed equally.
Study concept and design: Veenhoven, Wijmenga-Monsuur, de Melker, Sanders, Schouls, Berbers.
Acquisition of data: Spijkerman, Veenhoven, Elberse, van Gageldonk, Schouls, Berbers.
Analysis and interpretation of data: Spijkerman, Veenhoven, Elberse, van Gageldonk, Knol, de Melker, Sanders, Schouls, Berbers.
Drafting of the manuscript: Spijkerman, Veenhoven, Sanders, Schouls, Berbers.
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REFERENCES

1. O'Brien KL, Wolfson LJ, Watt JP, et al; Hib and Pneumococcal Global Burden of Disease Study Team. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet*. 2009;374(9693):893-902.

2. Vaccine schedule. European Centre for Disease Prevention and Control. http://vaccine-schedule.ecdc.europa.eu/Pages/Scheduler.aspx. Accessed August 13, 2013.

3. Ingels H, Rasmussen J, Andersen PH, et al; Danish Pneumococcal Surveillance Collaboration Group 2009-2010. Impact of pneumococcal vaccination in Denmark during the first 3 years after PCV introduction in the childhood immunization programme. *Vaccine*. 2012;30(26):3944-3950.

4. Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. *Lancet Infect Dis*. 2011;11(10):760-768.

5. Pilishvili T, Lexau C, Farley MM, et al; Active Bacterial Core Surveillance/Emerging Infections Program Network. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis*. 2010;201(1):32-41.

6. van Deursen AM, van Mens SP, Sanders EA, et al; Invasive Pneumococcal Disease Sentinel Surveillance Laboratory Group. Invasive pneumococcal disease and 7-valent pneumococcal conjugate vaccine: the Netherlands. *Emerg Infect Dis*. 2012;18(11):1729-1737.

7. Siber GR, Chang I, Baker S, et al. Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies. *Vaccine*. 2007;25(19):3816-3826.

8. PrabhuDas M, Adkins B, Gans H, et al. Challenges in infant immunity: implications for responses to infection and vaccines. *Nat Immunol*. 2011;12(3):189-194.

9. Brown GC, Volk VK, Gottshall RY, Kendrick PL, Anderson HD. Responses of infants to DTP-P vaccine used in nine injection schedules. *Public Health Rep*. 1964;79:585-602.

10. Goldblatt D, Southern J, Ashton L, et al. Immunogenicity of a reduced schedule of pneumococcal conjugate vaccine in healthy infants and correlates of protection for serotype 6B in the United Kingdom. *Pediatr Infect Dis J*. 2010;29(5):401-405.

11. Scott P, Rutjes AW, Bermetz L, et al. Comparing pneumococcal conjugate vaccine schedules based on 3 and 2 primary doses: systematic review and meta-analysis. *Vaccine*. 2011;29(52):9711-9721.

12. Rozenbaum MH, Sanders EA, van Hoek AJ, et al. Cost effectiveness of pneumococcal vaccination among Dutch infants: economic analysis of the seven valent pneumococcal conjugated vaccine and forecast for the 10 valent and 13 valent vaccines. *BMJ*. 2010;340:c2509.

13. Random.org. http://www.random.org. Accessed August 2, 2013.

14. van Houdt R, Koedijk FD, Bruisten SM, et al. Hepatitis B vaccination targeted at behavioural risk groups in the Netherlands: does it work? *Vaccine*. 2009;27(27):3530-3535.

15. Prymula R, Siegrist CA, Chlibek R, et al. Effect of prophylactic paracetamol administration at time of vaccination on febrile reactions and antibody responses in children: two open-label, randomised controlled trials. *Lancet*. 2009;374(9698):1339-1350.

16. Central Committee on Research Involving Human Subjects. http://www.ccmo-online.nl. Accessed August 2, 2013.

17. Elberse KE, Tcherniaeva I, Berbers GA, Schouls LM. Optimization and application of a multiplex bead-based assay to quantify serotype-specific IgG against *Streptococcus pneumoniae* polysaccharides: response to the booster vaccine after immunization with the pneumococcal 7-valent conjugate vaccine. *Clin Vaccine Immunol*. 2010;17(4):674-682.

18. de Voer RM, van der Klis FR, Schepp RM, Rijkers GT, Sanders EA, Berbers GA. Age-related immunity to meningococcal serogroup C vaccination: an increase in the persistence of IgG2 correlates with a decrease in the avidity of IgG. *PLoS One*. 2011;6(8):e23497.

19. Burton RL, Nahm MH. Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies. *Clin Vaccine Immunol*. 2006;13(9):1004-1009.

20. de Voer RM, Schepp RM, Versteegh FG, van der Klis FR, Berbers GA. Simultaneous detection of *Haemophilus influenzae* type b polysaccharide-specific antibodies and *Neisseria meningitidis* serogroup A, C, Y, and W-135 polysaccharide-specific antibodies in a fluorescent-bead-based multiplex immunoassay. *Clin Vaccine Immunol*. 2009;16(3):433-436.

21. van Gageldonk PG, van Schaijk FG, van der Klis FR, Berbers GA. Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to Bordetella pertussis, diphtheria and tetanus. *J Immunol Methods*. 2008;335(1-2):79-89.

22. Rodenburg GD, van Gils EJ, Veenhoven RH, et al. Comparability of antibody response to a booster dose of 7-valent pneumococcal conjugate vaccine in infants primed with either 2 or 3 doses. *Vaccine*. 2010;28(5):1391-1396.

23. Scott JA, Ojal J, Ashton L, Muhoro A, Burbidge P, Goldblatt D. Pneumococcal conjugate vaccine given shortly after birth stimulates effective antibody concentrations and primes immunological memory for sustained infant protection. *Clin Infect Dis*. 2011;53(7):663-670.

24. Pollard AJ, Perrett KP, Beverley PC. Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines. *Nat Rev Immunol*. 2009;9(3):213-220.

25. Melin M, Jarva H, Siira L, Meri S, Käyhty H, Väkeväinen M. *Streptococcus pneumoniae* capsular serotype 19F is more resistant to C3 deposition and less sensitive to opsonophagocytosis than serotype 6B. *Infect Immun*. 2009;77(2):676-684.

26. Spijkerman J, Prevaes SM, van Gils EJ, et al. Long-term effects of pneumococcal conjugate vaccine on nasopharyngeal carriage of *S. pneumoniae*, *S. aureus*, *H. influenzae* and *M. catarrhalis*. *PLoS One*. 2012;7(6):e39730.

27. van Gils EJ, Veenhoven RH, Hak E, et al. Effect of reduced-dose schedules with 7-valent pneumococcal conjugate vaccine on nasopharyngeal pneumococcal carriage in children: a randomized controlled trial. *JAMA*. 2009;302(2):159-167.

28. Simell B, Auranen K, Käyhty H, Goldblatt D, Dagan R, O'Brien KL; Pneumococcal Carriage Group. The fundamental link between pneumococcal carriage and disease. *Expert Rev Vaccines*. 2012;11(7):841-855.

29. Palmu AA, Jokinen J, Borys D, et al. Effectiveness of the ten-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV10) against invasive pneumococcal disease: a cluster randomised trial. *Lancet*. 2013;381(9862):214-222.

30. Scheifele DW, Halperin SA, Smith B, Ochnio J, Meloff K, Duarte-Monteiro D. Assessment of the compatibility of co-administered 7-valent pneumococcal conjugate, DTaP.IPV/PRP-T Hib and hepatitis B vaccines in infants 2-7 months of age. *Vaccine*. 2006;24(12):2057-2064.

31. Recommendations to assure the quality, safety and efficacy of pneumococcal conjugate vaccines. World Health Organization. http://www.who.int/biologicals/areas/vaccines/pneumo/Pneumo_final_23APRIL_2010.pdf. Accessed August 7, 2013.

32. Poehling KA, Talbot TR, Griffin MR, et al. Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. *JAMA*. 2006;295(14):1668-1674.

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