



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

The PIM study: Immunogenicity of the 13-valent polysaccharide pneumococcal conjugate vaccine

*Comparison of current schedule with
alternative and reduced immunisation
schedules*

RIVM report 230182001/2012

G.A.M. Berbers | L.M. Schouls



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Colophon

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Abstract

Good protection against pneumococcal disease using three instead of four vaccine doses

Pneumococci can cause serious invasive diseases such as meningitis and sepsis. Implementation of vaccination against pneumococci for young children at the age of 2, 3, 4 months (primary series) and at 11 months (booster dose) in 2006 has reduced this risk considerably. A clinical trial shows that vaccination at the age of 3, 5 and 11 months is as effective as, or even more effective than the current Dutch vaccination scheme. It is recommended to alter the current pneumococcal vaccination schedule.

Reduced burden and cost reduction

Administering fewer vaccine doses at a later age reduces the burden of the vaccination for the children. Moreover the reduced schedule results in a considerable cost reduction for the public health system. These findings are the results of the so-called PIM study ('Pneumokokken Iets Minder') performed by the RIVM, in cooperation with the Spaarne hospital and Wilhelmina children's hospital. The PIM study has been commissioned by the Ministry of Public Health.

Comparison of vaccination schedules

In this study, 400 healthy children were divided ad random into four groups. These four groups were vaccinated against 13 pneumococcal serotypes at 2, 3, 4 and 11 months, at 2, 4, 6 and 11 months, at 3, 5 and 11 months or at 2, 4 and 11 months.

Higher antibody levels after a 3-5 months' schedule

The amount of antibodies produced was measured in all children 1 month after the primary series, and at 12 months of age, 1 month after the booster. Antibody concentrations were also determined at the age of 8 months because the incidence of invasive pneumococcal disease was highest around that age before implementation of nationwide pneumococcal vaccination. After the full schedule at 12 months, all 4 vaccination schedules yielded protective antibody levels. However, after the primary series the 3-5 months' schedule resulted in higher antibody levels than the 2-3-4 months' schedule.

Keywords:

pneumococcal, vaccination, schedule, immunogenicity, antibodies

Rapport in het kort

Goede bescherming tegen pneumokokkenziekte kan met één prik minder

Sinds 2006 worden kinderen via het Rijksvaccinatieprogramma gevaccineerd tegen pneumokokken. Deze bacteriën kunnen ernstige ziekten veroorzaken, zoals hersenvliesontsteking en bloedvergiftiging. De kans hierop is door de vaccinatie flink verlaagd. De vaccinatie bestaat momenteel uit vier prikken die worden gegeven op de leeftijd van 2, 3 en 4 maanden (primaire serie) en 11 maanden (boostervaccinatie). Uit onderzoek blijkt nu dat de vaccinatie even effectief, of zelfs effectiever, is als de primaire serie van drie naar twee prikken wordt teruggebracht en deze in de derde en vijfde maand worden gegeven. Geadviseerd wordt om het huidige pneumokokken prikschema te wijzigen.

Minder belastend en kostenbesparend

Door minder en iets later te prikken zijn de vaccinaties voor de heel kleine kinderen minder belastend. Daarnaast levert dit schema een aanzienlijke kostenbesparing in de gezondheidszorg op (8 miljoen euro). Deze bevindingen zijn het resultaat van de zogeheten PIM-studie ('Pneumokokken Iets Minder'), die is uitgevoerd door het RIVM, in samenwerking met het Spaarne ziekenhuis en het Wilhelmina kinderziekenhuis. Opdrachtgever is het ministerie van VWS.

Vergelijking van vaccinatieschema's

In de PIM-studie kregen 400 gezonde kinderen een vaccinatie tegen 13 typen van de pneumokokkenbacterie. Zij zijn vervolgens ingedeeld in vier willekeurige groepen, die elk de vaccinaties op een ander tijdstip kreeg toegediend: met 2, 3, 4 en 11 maanden, met 2, 4, 6 en 11 maanden, met 3, 5 en 11 maanden, en met 2, 4 en 11 maanden.

Hogere antistofwaarden bij 3-5 maanden schema

Bij alle kinderen is vervolgens bepaald hoeveel antistoffen zij een maand na afloop van de primaire serie hadden gevormd, en een maand na de booster. Dit is ook gedaan op de leeftijd van 8 maanden, omdat vóór de invoering de pneumokokkenvaccinatie rond die leeftijd de meeste gevallen van pneumokokkenziekte voorkwamen. Na de primaire serie bleek de vaccinatie op 3 en 5 maanden hogere antistofwaarden te geven dan de vaccinatie op 2, 3 en 4 maanden. Op 12 maanden bleken alle vier de schema's voldoende antistoffen gegeven te hebben om te beschermen tegen pneumokokkenziekte.

Trefwoorden:

pneumokokken, vaccinatie, schema, immunogeniciteit, antistoffen

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Summary

Introduction

Pneumococci can cause serious invasive diseases, including meningitis, sepsis and pneumonia, predominantly in children and elderly. Pneumococcal conjugate vaccine protecting against seven serotypes of *S. pneumoniae* (PCV7) was implemented in the Dutch national immunisation program (NIP) in 2006. Children are vaccinated with PCV7 at 2, 3, 4 and 11 months of age.

Implementation of PCV7 more than halved the incidence of IPD among children below five years of age to 10 per 100,000 in 2009 and virtually no cases of IPD caused by the seven serotypes covered by PCV7 occurred thereafter in vaccinated children. Also in other age groups, the incidence of IPD caused by vaccine serotypes decreased due to herd immunity.

Although PCV7 was initially licensed to be administered as three primary doses, many countries use a schedule of only two primary doses. In addition, the interval between doses and the age of the first dose differs between countries. An immunisation schedule with fewer doses would obviously reduce the burden for children and costs. Furthermore, in this era where acceptance of vaccination is not self-evident anymore, reducing the number of doses could increase acceptance and, therefore, coverage of vaccination.

This report presents the results of the PIM ('Pneumokokken Iets Minder') study, a large randomised controlled trial (RCT), comparing the immunogenicity of 13-valent pneumococcal conjugate vaccine (PCV13) in four internationally used immunisation schedules.

Methods

A single-centre, phase 4, open-label randomised controlled parallel group trial was conducted in the Netherlands. In total, 400 healthy 'at term' born infants were randomized to receive PCV13 at: 2-4-6 months, 3-5 months, 2-3-4 months or 2-4 months with a booster dose at 11 months. All infants received DTaP-IPV-Hib vaccine at 2-3-4 and 11 months.

Blood samples were collected 1 month after the primary series, at 8 and 11 months, and 1 month after the booster dose. Pneumococcal serotype-specific IgG antibodies to the 13 vaccine pneumococcal serotypes were measured using a fluorescent bead-based multiplex immunoassay.

Differences between schedules in geometric mean concentrations (GMCs) of pneumococcal serotype-specific IgG antibodies and DTaP-IPV-Hib antibodies were analysed with ANOVA in the intention-to-treat population with Bonferroni correction for six multiple comparisons. Percentages of seroprotection were calculated using a cut-off value of 0.35 µg/ml for the pneumococcal IgG antibody levels and standard cut-off values for DTaP-IPV-Hib antibody levels. Non-inferiority of the alternative schedules against the Dutch 2-3-4 schedule was determined in the per-protocol population with Bonferroni correction for three multiple comparisons.

Results

After completion of the full vaccination series at 12 months, virtually no differences in IgG levels existed between the schedules and the seroprotection was almost 100% for all serotypes for all schedules. However, between the primary series and the booster dose at 11 months, some schedules were better

than others. The 2-4-6 schedule was clearly superior to the Dutch schedule for many serotypes. The 3-5 schedule was superior to the Dutch schedule on several serotypes, including 1, 3, 4, 5, 6A, 7F, 9V and 18C, but was just crossing the non-inferiority border for serotype 6B, 19F and 23F after the primary series. The 2-4 schedule was clearly inferior to the Dutch schedule for several serotypes. At 8 months of age, seroprotection was above 80% ($> 0.35 \mu\text{g/ml}$) for all serotypes in the 2-4-6 schedule and for almost all serotypes in the 3-5 schedule (serotype 4: 74%, serotype 6B: 39%). Seroprotection was less than 80% for serotype 1, 3, 4, 6B, 9V, 19F and 23F in the 2-3-4 schedule and for serotype 3, 4, 6B, 9V, 19A and 23F in the 2-4 schedule.

Conclusion

At 12 months of age, after the booster dose, all four immunisation schedules investigated in this study show adequate protection against IPD, and yielded similar antibody concentrations. However, in the period between the primary series and the booster dose, the 2-4-6 and 3-5 schedule are clearly better than the 2-3-4 and 2-4 schedule with respect to their induced IgG levels. When opting for a reduced dose schedule, the 3-5 schedule is the best choice offering a high level of seroprotection. Clinical effectiveness of the 3-5 schedule is confirmed by countries that already have implemented this schedule in their NIP. Any negative effects that the implementation of the 3-5 schedule might introduce are probably nullified by the already existing herd immunity. We recommend introduction of a 3-5 pneumococcal vaccination schedule which might be easily implemented when a 2-3-5 schedule for the primary vaccinations of the other components in the NIP is introduced leaving the start of the pertussis vaccinations at 2 months.

1 Introduction

Pneumococci can cause serious invasive diseases, including meningitis, sepsis and pneumonia, and are frequently causing otitis media. Pneumococcal disease predominantly occurs among children and the elderly. Before the implementation of pneumococcal vaccination, the incidence of invasive pneumococcal disease (IPD) was 36 per 100,000 among children below 2 years of age in the Netherlands (1). IPD resulted in 5% mortality and severe long-term sequelae in one-third of the patients (1). Pneumococcal conjugate vaccine protecting against seven of the more than 90 known serotypes (PCV7) was implemented in the Dutch national immunisation programme (NIP) in 2006. Children are vaccinated at 2, 3 and 4 months of age, and at 11 months of age with the so-called booster dose. In 2011, PCV7 was replaced by PCV10, which protects against 10 serotypes, for children born on or after March 1st 2011. After implementation of PCV7, the incidence of IPD more than halved among children below 5 years of age to 10 per 100,000 in 2009 with virtual eradication from carriage and disease by the 7 vaccine serotypes (2). In other age groups, the incidence of IPD caused by the vaccine serotypes decreased as well due to herd immunity.

Worldwide, it is estimated that more than 800,000 children under the age of five die from pneumococcal disease each year, making pneumococcal disease the number one vaccine-preventable killer (3). Many countries have therefore added PCV to their NIP. Although PCV7 was initially licensed to be administered as three primary doses, many countries use a schedule of only two primary doses. Also the interval between doses, the age at the first and last dose of the primary series, and the administration of a booster dose differs between countries (see website ECDC (4)). In most vaccination trials, the immunogenicity of the vaccine and vaccination schedules used is measured. An antibody concentration of 0.35 µg/ml is considered to represent a level that protects children against IPD and is referred to as the seroprotection. Several studies have investigated the impact of a full-dose (3+1) versus a reduced-dose (2+1) schedule of PCV7 on immunogenicity, demonstrating that a reduced number of doses in the primary series resulted in comparable antibody concentrations and seroprotection rates for all serotypes except for serotype 6B and 23F (5). Furthermore, these studies showed that a 2-month interval between two primary doses increased antibody responses (6). Moreover, starting the first vaccination dose of the primary series one month later seemed beneficial in terms of physiological maturity of the immune system of infants but at the possible cost that protection is achieved at a slightly later age (7). Although a number of studies on vaccination schedules are available, none of these studies compared more than two immunisation schedules in a single study and in a single region or country, which is needed to assess the relative contribution of all aspects of different immunisation schedules.

An immunisation schedule with fewer doses would obviously reduce the burden for children and would reduce costs. Furthermore, in this era where acceptance of vaccination is not self-evident anymore, reducing the number of doses could contribute to vaccination acceptance and, consequently, coverage of vaccination. When the Dutch Health Council advised about implementation of pneumococcal vaccination, the council judged there was insufficient scientific evidence that a reduced-dose schedule was as effective as a full-dose schedule and therefore a full-dose schedule was implemented. The Dutch Health Council recommended

investigating the effects of reduced immunisation schedules in comparison with the current schedule.

This report presents the results of the PIM ('Pneumokokken Iets Minder') study, a large randomised controlled trial (RCT), comparing immunogenicity of four internationally used immunisation schedules (two full-dose and two reduced-dose schedules) using the recently licensed 13-valent pneumococcal conjugate vaccine (PCV13; Prevenar 13™; Pfizer, Inc., New York, USA) for protection against *S. pneumoniae*.

2 Methods

2.1 Study design

A single-centre, phase 4, randomised controlled parallel group trial was conducted in the Netherlands to investigate the effects of four different primary immunisation schedules on the immune response against the serotypes included in PCV13 (i.e. serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F all conjugated to CRM-197, a non-toxic variant of diphtheria toxin). In total, 400 infants were randomly assigned (1:1:1:1) to receive PCV13 either at 2, 4 and 6 ('USA schedule'), at 3 and 5 ('Scandinavian schedule'), at 2, 3 and 4 ('Dutch schedule'), or at 2 and 4 months of age ('UK schedule'). All infants received a booster dose of PCV-13 at 11 months of age. The PCV13 vaccines were kindly provided by Pfizer. According to the Dutch NIP, DTaP-IPV-Hib (PediaceTM, Sanofi Pasteur MSD, Lyon, France) was administered at 2, 3, 4 and 11 months of age. The first dose was given at 60 (-/+7) days and the interval between subsequent monthly vaccine doses was designed to be 28 (-/+7) days. The booster vaccine dose was actually administered at 11.5 months (-/+ 7 days) and the post booster sample at 12.5 months (-/+ 7 days) but for simplicity we have used 11 and 12 months throughout the report. In the remainder of the report complete schedules including the booster at 11 months are designated as the 2-3-4, 2-4-6, 3-5 and 2-4 schedules. The study design with all clinical handlings is illustrated in Figure 1. Vaccines were administered intramuscularly in the right (PCV-13) or left (DTaP-IPV-Hib) anterolateral thigh. Randomization was performed by random number generator (www.random.org) using block randomization with randomly varying block size. Allocations were concealed in sequentially numbered envelopes that were opened during a home visit after enrolment of the infant. This was an open-label study; i.e. study staff members and parents were aware of the child's allocated immunisation schedule, but laboratory personnel assessing immunogenicity endpoints was blinded.

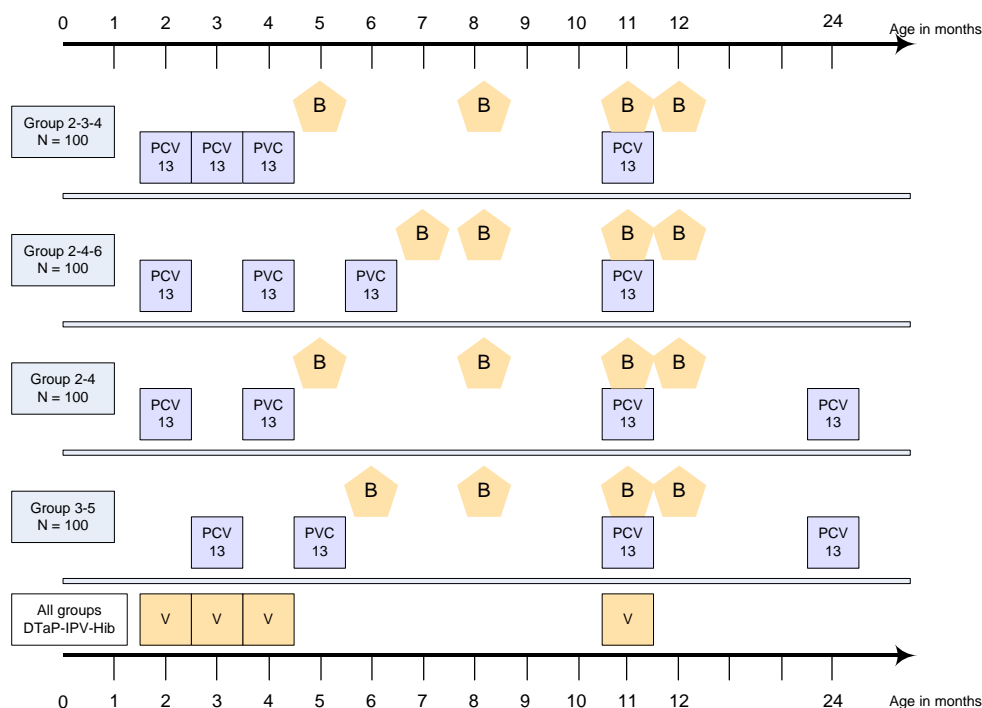


Figure 1. Overview of vaccination and blood sampling time points for the four different vaccination schedules
 PCV13, vaccination with the 13-valent pneumococcal vaccine; B, blood sampling; V, vaccination with the DTaP-IPV-Hib combination vaccine.

2.2 Study subjects

Parents of newborns eligible for routine childhood immunisation, excluding those eligible for Hepatitis B vaccination (i.e. children with at least one parent born in a middle or high endemic country), were offered the opportunity to participate. Healthy infants born after a gestation period of at least 37 weeks, not yet having received any infant vaccination, and living inside the study area were eligible. The study area covered the province of Noord-Holland, excluding the city of Amsterdam and the Gooi region. Exclusion and/or elimination criteria were any known primary or secondary immunodeficiency, bleeding disorders, receipt of immunoglobulin or blood products and a language barrier. At each visit, a brief survey was obtained from parents for baseline demographics, occurring illnesses, and medication usage including prophylactic paracetamol administration as this may significantly reduce antibody responses (14). An additional fourth PCV13 vaccination at 24 months of age was offered free of charge to all infants who received a 2+1 schedule. Participants did not receive any financial compensation. An acknowledged independent national ethics committee (Centrale Commissie Mensgebonden Onderzoek, available at: <http://www.ccmo-online.nl>) approved the study protocol (NTR2316). Written informed consent was obtained from each infant's parent(s)/guardian(s) before enrolment. The study was performed in accordance with European guidelines for Good Clinical Practice, which includes provisions of the declaration of Helsinki.

2.3 Serological analyses

Venous blood samples of approximately 2 ml were collected from all participants at four different time points: 1 month (28 \pm 7 days) after the primary series (i.e. post-primary), at 8 months of age (-7/+14 days), pre-booster, and 1 month (28 \pm 7 days) after the booster dose (i.e. post-booster). Blood sampling was postponed in case of fever $> 38.5^{\circ}\text{C}$ within 24 hours before the planned visit to exclude possible interference of cellular immune responses. Sera were stored at -80°C and sent afterwards in bulk to the National Institute for Public Health and the Environment (Bilthoven, The Netherlands) for assessment of immune responses. Pneumococcal serotype-specific IgG antibodies to the 13 vaccine pneumococcal serotypes were measured using a fluorescent bead-based multiplex immuno assay (MIA) as described previously (8). Responses to routine DTPa-IPV-Hib antigens were determined according to availability of serum by using a MIA (for Diphtheria, Tetanus, Pertussis, and *Haemophilus influenzae* type b) and a microneutralization test (for Poliovirus) adopted from WHO guidelines (9-11) (16;17; extra ref). All assays were performed in duplicate.

2.4 Statistical analysis

2.4.1 Sample size calculation

Taking correction for six multiple tests into account, a sample size of 80 evaluable subjects was needed to detect a 2-fold difference (ratio of 1.90 or 0.52) in the geometric mean concentrations (GMCs) of antibodies directed against polysaccharide 6B, the serotype with the highest variance in antibody levels, with 80% power and a significance level of 5% (see protocol NTR2316). Estimating that in up to 20% of infants, an insufficient blood sample would be available for serological testing, 100 subjects per group were included in this study.

2.4.2 Superiority analyses

The superiority analyses were primarily performed on the intention-to-treat (ITT) population, which means that all available data from all participants allocated to a treatment group were analysed according to the assigned intervention. ITT analysis is the preferred method of analysis for superiority analyses, as it generally gives more conservative effect estimates than per-protocol (PP) analysis. For each randomization group, pneumococcal serotype-specific GMCs and two-sided 95% confidence intervals (CIs) were calculated at all time-points. Differences between groups were analyzed using ANOVA and p-values were adjusted for six multiple comparisons using Bonferroni correction (3+2+1=6 comparisons were made between the four vaccination schedules). Percentages of seroprotection were calculated using a cut-off value of $0.35 \mu\text{g/ml}$ for the pneumococcal antibody levels at all time-points, and a cut-off value of $1.0 \mu\text{g/ml}$ for antibody concentrations measured 1 month after the booster dose. GMCs and two-sided 95% confidence intervals (CIs) were also calculated for DTPa-IPV-Hib antigens and standard correlates of protection were used to calculate percentages of seroprotection (diphtheria and tetanus: $\geq 0.1 \text{ IU/ml}$, polio: titer ≥ 8 , *H. Influenzae* type b: $\geq 0.15 \mu\text{g/ml}$, pertussis toxin: $\geq 20 \text{ EU/ml}$ arbitrary cut-off).

2.4.3 Non-inferiority analyses

The non-inferiority analyses were primarily performed on the PP population, including only infants who received the allocated intervention and complied with all pre-specified vaccination and blood sampling intervals in absence of any

elimination criterion. In contrast to superiority analyses, PP analysis is the preferred method of analysis for non-inferiority, as PP analysis generally gives more conservative results regarding non-inferiority than ITT analysis. Pneumococcal serotype-specific GMC ratios and one-sided CIs were calculated for each immunisation schedule with the Dutch schedule (2, 3, 4 months) as the reference. As the three alternative schedules were compared with the Dutch schedule with respect to non-inferiority, three multiple comparisons were done, and therefore Bonferroni correction for three (rather than six in the superiority analyses) multiple comparisons was performed. To adjust for three multiple comparisons using Bonferroni correction, 98.3% CIs were calculated rather than the usual 95% CIs. The non-inferiority margin was defined as a GMC ratio of 0.5 (see protocol NTR2316), meaning that if the lower limit of the CI around the GMC ratio was larger than 0.5 non-inferiority was assigned.

2.4.4 *Longitudinal analyses*

To evaluate the decrease of antibody concentrations after the primary series up to the booster dose, the difference in log₁₀ transformed antibody concentrations between 8 months of age and post-primary (first time interval), and between pre-booster and 8 months of age (second time interval) was calculated in children with results available at all three time points (complete case analysis). Paired t-tests for all serotypes in each randomisation group were performed to test for differences in decline between the first and second time interval.

Data were analysed using SPSS 19.0. and a p-value of < 0.05 was considered statistically significant.

3 Results

3.1 Study subjects

Between 30 June 2010 and 25 January 2011, 400 infants were enrolled of whom 396 (99%) the study was completed (Figure 2). From the 1590 blood sampling attempts, in 1440 cases (90%) sufficient blood could be obtained and results were included in the ITT analyses. Of these, 1427 (99%) could also be included in the PP analyses. There were no marked differences in baseline characteristics and age at visits between the randomisation groups (Table 1). Paracetamol was only rarely administered to infants as a prophylactic before each vaccination (<2% times per randomisation group).

Table 1. Characteristics of participating children

	2-4-6 schedule	3-5 schedule	2-3-4 schedule	2-4 schedule
Participants (n)	100	100	100	100
Male sex (%)	57•0	53•0	55•0	49•0
Birth Weight (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 yra (%)	47•0	53•0	43•0	48•0
Daycare attendance ^b (%)	61•0	59•0	49•5	48•0
Age, mean (SD), mo:				
at visit 2 mo	1•9 (0•1)	1•9 (0•1)	1•9 (0•1)	1•9 (0•1)
at visit 3 mo	2•9 (0•2)	2•9 (0•1)	2•9 (0•1)	2•9 (0•2)
at visit 4 mo	3•9 (0•2)	3•9 (0•1)	3•9 (0•2)	3•9 (0•2)
at visit 5 mo		4•9 (0•2)	4•9 (0•2)	4•9 (0•3)
at visit 6 mo	5•9 (0•2)	5•9 (0•2)		
at visit 7 mo	6•9 (0•2)			
at visit 8 mo	8•1 (0•1)	8•1 (0•1)	8•1 (0•1)	8•1 (0•1)
at visit 11 mo	11•5 (0•2)	11•5 (0•2)	11•5 (0•2)	11•5 (0•2)
at visit 12 mo	12•5 (0•2)	12•4 (0•2)	12•5 (0•2)	12•5 (0•2)

^a Information was asked at enrolment. ^b Defined as at least four continuous hours per week with at least one child < 5 yr of age from a different family, asked at 4 months of age.

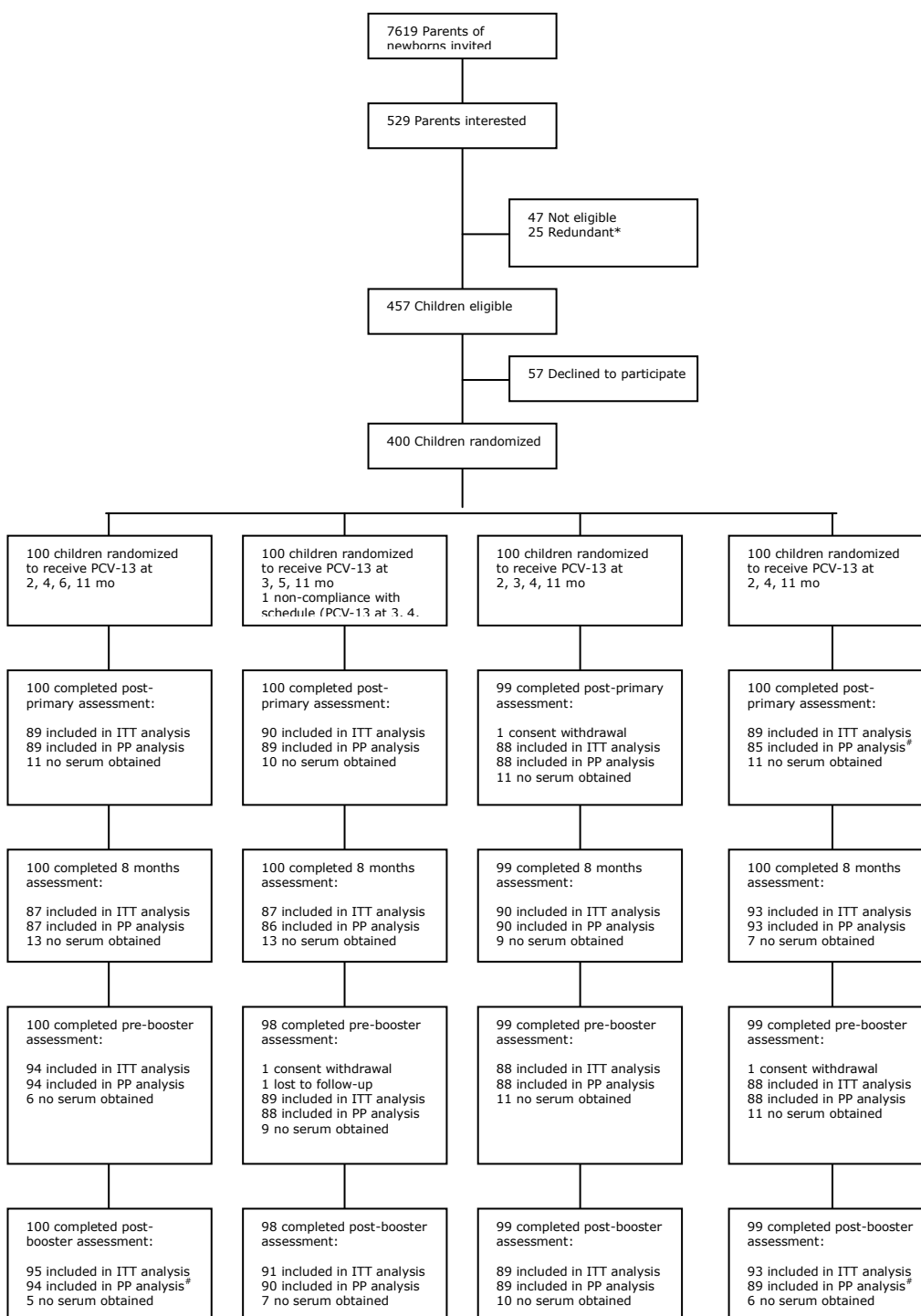


Figure 2. Flow chart

* Parents of children interested in participating in the study were considered redundant because the enrolment target had already been achieved and informed consent procedure was cancelled. # Samples are excluded from PP analysis due to non-compliance with blood sampling schedule. Abbreviations: PCV-13, 13-valent pneumococcal conjugate vaccine (Pfizer, Inc.); ITT, intention to treat; PP, per-protocol.

3.2 Immunogenicity of PCV13

3.2.1 Superiority analyses

Figure 3A-C and Table 2A-D present GMCs and seroprotection rates for each randomization group per serotype at each time-point, respectively. Compared with the Dutch immunisation schedule (2-3-4), the 2-4-6 schedule was superior for most serotypes after the primary series (except for serotype 3, 9V, 19A, and 19F), at 8 months of age and before the booster dose (except for serotype 14, 19A and 19F) (see also summary Supplementary Table 1A-D). The 3-5 schedule was superior to the Dutch schedule on several serotypes (serotype 1, 4, 5, 6A and 7F after primary series, serotype 1, 3, 4, 5, 6A, 7F, 9V and 18C at 8 months of age, serotype 1, 3, 4, 5, 6A and serotype 7F before the booster dose). The 2-4 schedule was only superior to the Dutch schedule for serotype 1 and even inferior for serotype 3, 6B, 19A, 19F and 23F at several time-points. After the booster dose, none of the alternative schedules was superior or inferior to the Dutch schedule except for the 2-4-6 schedule which was superior for serotypes 18C and 23F, and the 3-5 schedule which was superior for serotype 1 (Supplementary Table 1D).

When the alternative schedules were mutually compared, the 2-4-6 schedule was superior to the 3-5 schedule on several serotypes (serotype 6A, 6B and 23F after the primary series, serotype 5, 6A, 6B, 7F, 9V, 18C and 19F at 8 months of age, and serotype 6A, 6B, 9V and 18C before the booster dose) and inferior on serotype 3 (after primary series) and 23F (at 8 months of age and before the booster dose). After the booster dose, the 2-4-6 and 3-5 schedules were comparable except for serotype 1 where the 3-5 schedule was superior to the 2-4-6 schedule. The 2-4 schedule was inferior to the 2-4-6 and 3-5 schedule for almost all serotypes after the primary series, and at 8 and 11 months. After the booster dose, the 2-4 schedule was inferior to the 2-4-6 schedule for serotypes 6B, 18C and 23F, and inferior to the 3-5 schedule for serotype 1.

At 8 months of age, seroprotection was above 80% (cut-off > 0.35 µg/ml) for all serotypes in the 2-4-6 schedule and for almost all serotypes in the 3-5 schedule (serotype 4: 74%, serotype 6B: 39%). Seroprotection was less than 80% for serotype 1, 3, 4, 6B, 9V, 19F and 23F in the 2-3-4 and for serotype 3, 4, 6B, 9V, 19A and 23F for the 2-4 schedule (Table 2B). After the booster dose, seroprotection at 12 months of age was almost 100% for all serotypes for all schedules. Using the higher cut-off value (> 1.0 µg/ml), seroprotection was above 80% for all schedules and all serotypes, except for serotype 3 (Table 2D). Supplementary Figure 1A-D shows reverse cumulative distribution plots in which the influence of different cut-off values on the percentage of seroprotection for the four vaccination schedules is illustrated per serotype. Analyses in the PP population yielded virtually identical results (data not shown).

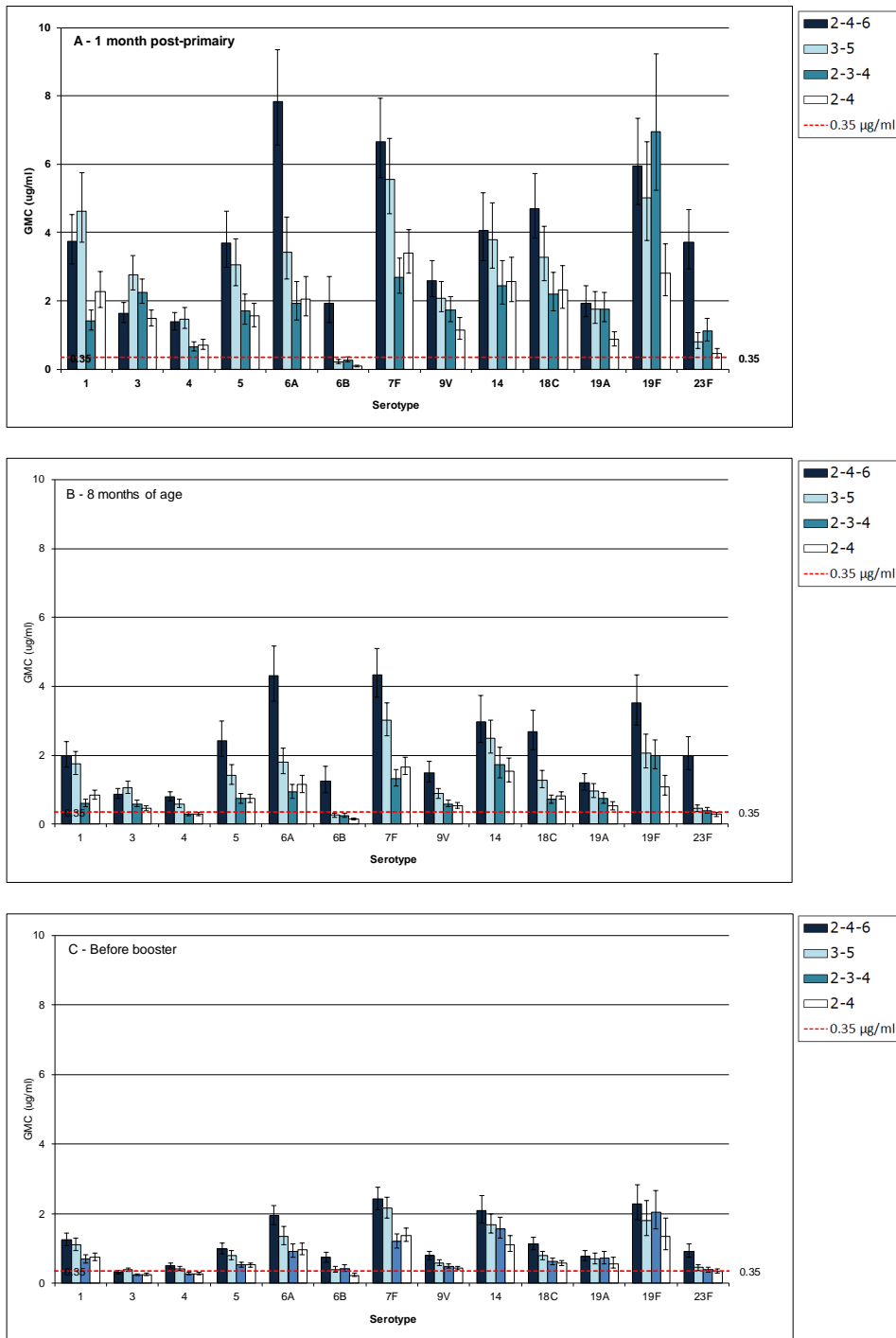


Figure 3A. Pneumococcal serotype-specific antibody GMCs measured at different time point
 Panel A, GMCs one month after the primary vaccination series; panel B, GMCs at 8 months of age; panel C, GMCs before the booster dose of PCV13.

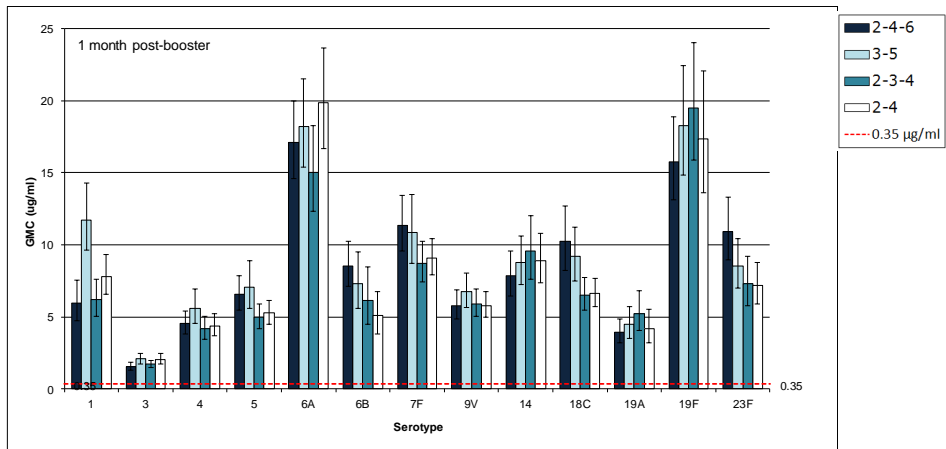


Figure 3B. Pneumococcal serotype-specific antibody GMCs measured one month after the booster dose of PCV13
 Note that the scale of the Y-axis differs from that of figure 3A.

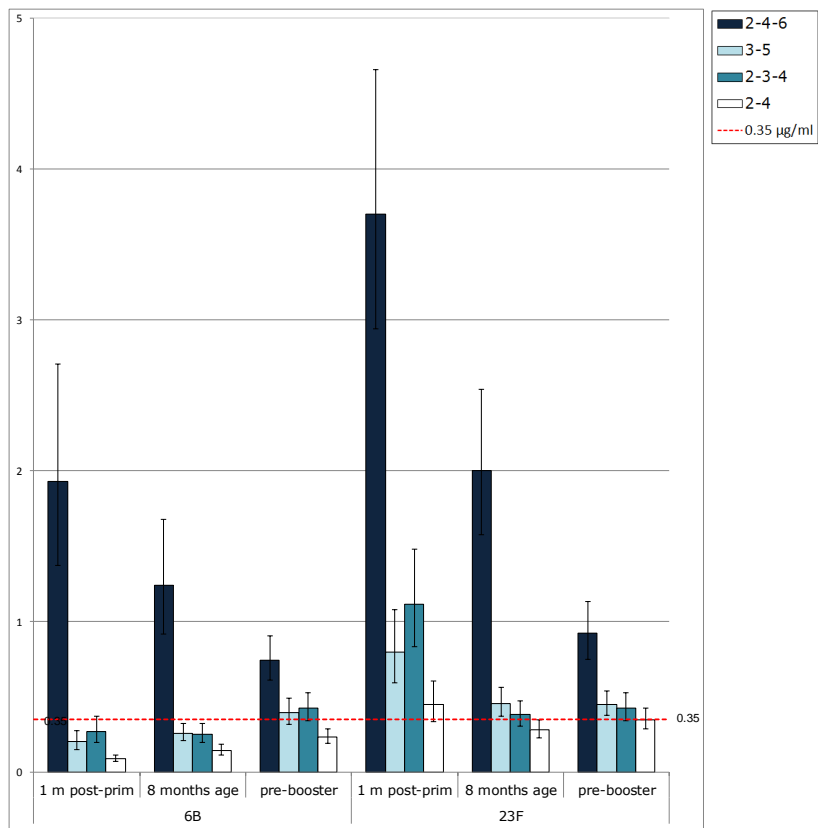


Figure 3C. Detailed view of the antibody GMCs against serotypes 6B and 23F, one month after the primary series, at the age of months and pre-booster
 Note that the scale of the Y-axis has been adjusted to visualize lower GMCs.

Table 2A. Pneumococcal serotype-specific antibody GMCs and antibody concentration > 0.35 ug/ml measured 1 month after the primary series in intention-to-treat population with p-values for superiority

Serotype	2-4-6 schedule (n=89)		3-5 schedule (n=90)		2-3-4 schedule (n=88)		2-4 schedule (n=89)		P-value ^a					
	GMC (95% CI)	%> 0.35	GMC (95% CI)	%> 0.35	GMC (95% CI)	%> 0.35	GMC (95% CI)	%> 0.35	2-4-6 vs. 3-5	2-4-6 vs. 2-3-4	2-4-6 vs. 2-4	3-5 vs. 2-3-4	3-5 vs. 2-4	2-3-4 vs. 2-4
1	3.73 (3.07-4.52)	100	4.62 (3.72-5.74)	97	1.40 (1.13-1.74)	96	2.27 (1.79-2.87)	93	0.953	< 0.001	0.08	< 0.001	< 0.001	0.011
3	1.62 (1.35-1.95)	98	2.76 (2.30-3.32)	99	2.25 (1.92-2.64)	99	1.48 (1.27-1.72)	96	< 0.001	0.046	1.000	0.537	< 0.001	0.004
4	1.38 (1.15-1.66)	94	1.46 (1.18-1.80)	93	0.65 (0.53-0.79)	76	0.70 (0.57-0.87)	82	1.000	< 0.001	< 0.001	< 0.001	< 0.001	1.000
5	3.70 (2.97-4.61)	98	3.05 (2.45-3.80)	97	1.69 (1.31-2.19)	90	1.55 (1.25-1.92)	92	1.000	< 0.001	< 0.001	0.002	< 0.001	1.000
6A	7.84 (6.56-9.36)	100	3.42 (2.64-4.44)	93	1.91 (1.44-2.55)	91	2.05 (1.55-2.71)	87	< 0.001	< 0.001	< 0.001	0.009	0.030	1.000
6B	1.93 (1.37-2.71)	88	0.20 (0.15-0.27)	30	0.27 (0.20-0.37)	44	0.09 (0.07-0.11)	11	< 0.001	< 0.001	< 0.001	1.000	0.001	< 0.001
7F	6.66 (5.61-7.92)	100	5.54 (4.55-6.75)	99	2.69 (2.22-3.26)	99	3.39 (2.81-4.08)	97	1.000	< 0.001	< 0.001	< 0.001	0.002	0.511
9V	2.59 (2.12-3.17)	98	2.08 (1.68-2.56)	96	1.72 (1.39-2.13)	96	1.15 (0.88-1.50)	80	1.000	0.065	< 0.001	1.000	0.001	0.078
14	4.05 (3.18-5.15)	96	3.79 (2.96-4.86)	94	2.45 (1.89-3.17)	92	2.55 (1.98-3.28)	90	1.000	0.029	0.057	0.084	0.153	1.000
18C	4.68 (3.84-5.72)	99	3.28 (2.57-4.18)	94	2.20 (1.71-2.82)	93	2.31 (1.77-3.03)	91	0.229	< 0.001	< 0.001	0.123	0.259	1.000
19A	1.93 (1.53-2.43)	92	1.74 (1.34-2.26)	88	1.75 (1.38-2.23)	90	0.86 (0.68-1.09)	76	1.000	1.000	< 0.001	1.000	< 0.001	< 0.001
19F	5.94 (4.81-7.33)	100	5.01 (3.77-6.67)	94	6.95 (5.24-9.22)	98	2.81 (2.16-3.65)	97	1.000	1.000	< 0.001	0.482	0.012	< 0.001
23F	3.70 (2.94-4.66)	97	0.80 (0.59-1.08)	68	1.11 (0.83-1.48)	83	0.45 (0.33-0.60)	58	< 0.001	< 0.001	< 0.001	0.586	0.025	< 0.001

Abbreviations: GMC, geometric mean concentration; CI, confidence interval ^a Differences in GMCs between groups were analyzed using ANOVA. P-values were adjusted for six multiple comparisons using Bonferroni correction.

Table 2B. Pneumococcal serotype-specific antibody GMCs and antibody concentration > 0.35 ug/ml measured at 8 months of age in intention-to-treat population with p-values for superiority

Serotype	2-4-6 schedule (n=87)		3-5 schedule (n=87)		2-3-4 schedule (n=90)		2-4 schedule (n=93)		P-value ^a					
	GMC (95% CI)	%> 0.35	GMC (95% CI)	%> 0.35	GMC (95% CI)	%> 0.35	GMC (95% CI)	%> 0.35	2-4-6 vs. 3-5	2-4-6 vs. 2-3-4	2-4-6 vs. 2-4	3-5 vs. 2-3-4	3-5 vs. 2-4	2-3-4 vs. 2-4
1	1.99 (1.64-2.40)	98	1.74 (1.44-2.11)	95	0.60 (0.51-0.71)	79	0.85 (0.72-0.99)	87	1.000	< 0.001	< 0.001	< 0.001	< 0.001	0.041
3	0.87 (0.73-1.04)	93	1.06 (0.89-1.26)	93	0.59 (0.50-0.69)	78	0.45 (0.39-0.52)	59	0.551	0.004	< 0.001	< 0.001	< 0.001	0.115
4	0.79 (0.67-0.95)	84	0.59 (0.48-0.72)	72	0.29 (0.24-0.34)	39	0.29 (0.26-0.34)	36	0.090	< 0.001	< 0.001	< 0.001	< 0.001	1.000
5	2.42 (1.96-2.99)	94	1.41 (1.15-1.73)	94	0.73 (0.60-0.90)	83	0.74 (0.63-0.86)	80	0.001	< 0.001	< 0.001	< 0.001	< 0.001	1.000
6A	4.30 (3.58-5.18)	100	1.80 (1.46-2.21)	95	0.93 (0.75-1.16)	90	1.14 (0.92-1.42)	85	< 0.001	< 0.001	< 0.001	< 0.001	0.013	0.972
6B	1.24 (0.91-1.68)	84	0.26 (0.20-0.32)	39	0.25 (0.20-0.32)	46	0.14 (0.11-0.18)	22	< 0.001	< 0.001	< 0.001	1.000	0.007	0.010
7F	4.34 (3.69-5.10)	100	3.01 (2.57-3.53)	100	1.33 (1.11-1.58)	94	1.66 (1.43-1.93)	97	0.011	< 0.001	< 0.001	< 0.001	< 0.001	0.286
9V	1.49 (1.23-1.81)	97	0.88 (0.74-1.04)	86	0.59 (0.50-0.70)	79	0.54 (0.46-0.63)	76	< 0.001	< 0.001	< 0.001	0.007	0.001	1.000
14	2.98 (2.38-3.73)	95	2.49 (2.05-3.02)	95	1.74 (1.35-2.23)	91	1.53 (1.22-1.93)	91	1.000	0.005	< 0.001	0.158	0.016	1.000
18C	2.68 (2.17-3.31)	98	1.28 (1.06-1.55)	93	0.72 (0.60-0.85)	80	0.83 (0.73-0.94)	90	< 0.001	< 0.001	< 0.001	< 0.001	0.004	1.000
19A	1.20 (0.98-1.47)	87	0.95 (0.77-1.17)	81	0.74 (0.60-0.91)	84	0.52 (0.42-0.66)	62	0.775	0.009	< 0.001	0.582	0.001	0.148
19F	1.99 (1.64-2.40)	98	1.74 (1.44-2.11)	95	0.60 (0.51-0.71)	79	0.85 (0.72-0.99)	87	0.009	0.003	< 0.001	1.000	0.001	0.002
23F	0.87 (0.73-1.04)	93	1.06 (0.89-1.26)	93	0.59 (0.50-0.69)	78	0.45 (0.39-0.52)	59	< 0.001	< 0.001	< 0.001	1.000	0.012	0.298

Abbreviations: GMC, geometric mean concentration; CI, confidence interval ^a Differences in GMCs between groups were analyzed using ANOVA. P-values were adjusted for six multiple comparisons using Bonferroni correction.

Table 2C. Pneumococcal serotype-specific antibody GMCs and antibody concentration > 0.35ug/ml measured before the booster dose in intention-to-treat population with p-values for superiority

Serotype	2-4-6 schedule (n=94)		3-5 schedule (n=89)		2-3-4 schedule (n=88)		2-4 schedule (n=88)		P-value ^a					
	GMC (95% CI)	%> 0.35	GMC (95% CI)	%> 0.35	GMC (95% CI)	%> 0.35	GMC (95% CI)	%> 0.35	2-4-6 vs. 3-5	2-4-6 vs. 2-3-4	2-4-6 vs. 2-4	3-5 vs. 2-3-4	3-5 vs. 2-4	2-3-4 vs. 2-4
1	1.24 (1.07-1.43)	96	1.11 (0.95-1.31)	98	0.69 (0.58-0.82)	85	0.76 (0.66-0.86)	85	1.000	< 0.001	< 0.001	< 0.001	0.003	1.000
3	0.32 (0.28-0.37)	38	0.38 (0.33-0.44)	47	0.24 (0.22-0.27)	21	0.25 (0.21-0.29)	22	0.438	0.031	0.075	< 0.001	< 0.001	1.000
4	0.51 (0.44-0.58)	69	0.41 (0.35-0.48)	55	0.28 (0.25-0.32)	35	0.28 (0.24-0.31)	34	0.230	< 0.001	< 0.001	0.001	< 0.001	1.000
5	0.99 (0.84-1.16)	92	0.80 (0.68-0.94)	85	0.53 (0.46-0.61)	71	0.52 (0.46-0.59)	71	0.267	< 0.001	< 0.001	0.001	< 0.001	1.000
6A	1.93 (1.68-2.23)	100	1.35 (1.11-1.64)	94	0.92 (0.75-1.13)	90	0.97 (0.82-1.16)	88	0.031	< 0.001	< 0.001	0.019	0.070	1.000
6B	0.74 (0.61-0.90)	80	0.39 (0.32-0.49)	52	0.42 (0.34-0.52)	65	0.23 (0.19-0.29)	35	< 0.001	0.001	< 0.001	1.000	0.003	0.001
7F	2.43 (2.13-2.77)	100	2.16 (1.87-2.48)	99	1.20 (1.00-1.43)	97	1.38 (1.20-1.57)	96	1.000	< 0.001	< 0.001	< 0.001	< 0.001	1.000
9V	0.79 (0.68-0.91)	86	0.59 (0.51-0.67)	79	0.49 (0.43-0.55)	76	0.43 (0.38-0.49)	63	0.014	< 0.001	< 0.001	0.304	0.007	1.000
14	2.09 (1.73-2.53)	96	1.69 (1.44-1.99)	96	1.57 (1.30-1.90)	97	1.12 (0.91-1.38)	89	0.656	0.191	< 0.001	1.000	0.012	0.068
18C	1.14 (0.97-1.33)	94	0.79 (0.69-0.92)	91	0.62 (0.54-0.71)	78	0.58 (0.51-0.66)	82	0.002	< 0.001	< 0.001	0.102	0.013	1.000
19A	0.78 (0.65-0.93)	82	0.70 (0.56-0.86)	78	0.72 (0.56-0.92)	81	0.57 (0.43-0.75)	61	1.000	1.000	0.328	1.000	1.000	0.943
19F	1.24 (1.07-1.43)	96	1.11 (0.95-1.31)	98	0.69 (0.58-0.82)	85	0.76 (0.66-0.86)	85	1.000	1.000	0.046	1.000	0.849	0.226
23F	0.32 (0.28-0.37)	38	0.38 (0.33-0.44)	47	0.24 (0.22-0.27)	21	0.25 (0.21-0.29)	22	< 0.001	< 0.001	< 0.001	1.000	0.371	1.000

Abbreviations: GMC, geometric mean concentration; CI, confidence interval ^a Differences in GMCs between groups were analyzed using ANOVA. P-values were adjusted for six multiple comparisons using Bonferroni correction.

Table 2D. Pneumococcal serotype-specific antibody GMCs and antibody concentration > 1.0 ug/ml measured 1 month after the booster dose in intention-to-treat population with p-values for superiority

Serotype	2-4-6 schedule (n=95)		3-5 schedule (n=91)		2-3-4 schedule (n=89)		2-4 schedule (n=93)		P-value ^a		2-4-6 vs. 3-5	2-4-6 vs. 2-3-4	2-4-6 vs. 2-4	3-5 vs. 2-3-4	3-5 vs. 2-4	2-3-4 vs. 2-4		
	GMC (95% CI)	%> 0.35	%> 1.0	GMC (95% CI)	%> 0.35	%> 1.0	GMC (95% CI)	%> 0.35	%> 1.0	GMC (95% CI)							%> 0.35	%> 1.0
1	5.95 (4.71-7.53)	99	98	11.74 (9.63-14.31)	100	99	6.18 (5.04-7.58)	100	96	7.81 (6.56-9.30)	100	100	< 0.001	1.000	0.360	< 0.001	0.033	0.660
3	1.56 (1.31-1.86)	95	71	2.07 (1.74-2.46)	100	79	1.70 (1.48-1.96)	99	79	2.05 (1.73-2.43)	98	77	0.097	1.000	0.116	0.613	1.000	0.705
4	4.53 (3.78-5.42)	100	96	5.59 (4.53-6.90)	99	95	4.17 (3.46-5.03)	100	93	4.38 (3.69-5.21)	99	98	0.684	1.000	1.000	0.185	0.419	1.000
5	6.54 (5.45-7.84)	100	100	7.05 (5.58-8.91)	99	96	4.96 (4.16-5.91)	99	99	5.26 (4.50-6.14)	100	99	1.000	0.245	0.615	0.059	0.176	1.000
6A	17.09 (14.61-19.98)	100	99	18.22 (15.42-21.54)	100	100	15.02 (12.35-18.27)	100	99	19.87 (16.68-23.67)	100	100	1.000	1.000	1.000	0.737	1.000	0.150
6B	8.53 (7.13-10.22)	100	100	7.29 (5.59-9.50)	98	92	6.15 (4.48-8.45)	94	92	5.09 (3.84-6.74)	97	91	1.000	0.496	0.034	1.000	0.339	1.000
7F	11.36 (9.59-13.46)	100	100	10.86 (8.73-13.51)	99	99	8.73 (7.42-10.26)	100	100	9.07 (7.89-10.44)	100	100	1.000	0.208	0.411	0.498	0.897	1.000
9V	5.77 (4.86-6.85)	100	100	6.74 (5.68-8.01)	100	100	5.91 (5.04-6.92)	100	100	5.79 (4.96-6.75)	100	100	1.000	1.000	1.000	1.000	1.000	1.000
14	7.86 (6.44-9.58)	99	97	8.76 (7.23-10.61)	98	98	9.54 (7.58-12.01)	97	97	8.91 (7.35-10.81)	100	99	1.000	1.000	1.000	1.000	1.000	1.000
18C	10.23 (8.22-12.72)	99	99	9.19 (7.51-11.25)	99	99	6.50 (5.44-7.76)	100	99	6.60 (5.68-7.68)	99	99	1.000	0.005	0.007	0.068	0.088	1.000
19A	3.92 (3.17-4.84)	99	90	4.48 (3.52-5.71)	99	89	5.25 (4.05-6.79)	97	87	4.19 (3.16-5.55)	95	84	1.000	0.602	1.000	1.000	1.000	1.000
19F	15.74 (13.12-18.88)	100	100	18.27 (14.86-22.45)	100	100	19.54 (15.87-24.05)	100	100	17.35 (13.64-22.08)	100	98	1.000	0.897	1.000	1.000	1.000	1.000
23F	10.93 (8.96-13.32)	100	100	8.53 (7.00-10.41)	100	98	7.29 (5.78-9.19)	99	97	7.17 (5.87-8.77)	99	98	0.558	0.039	0.025	1.000	1.000	1.000

Abbreviations: GMC, geometric mean concentration; CI, confidence interval ^a Differences in GMCs between groups were analyzed using ANOVA. P-values were adjusted for 6 multiple comparisons using Bonferroni correction.

3.2.2 *Non-inferiority analyses*

Figure 3A-D presents GMC ratios (GMC of alternative schedule divided by GMC of Dutch schedule) with confidence intervals to assess non-inferiority of the alternative schedules compared with the Dutch schedule. If the lower limit of the confidence interval is larger than the pre-defined non-inferiority margin of 0.5, the alternative schedule is non-inferior to the Dutch schedule. Note that superiority cannot be directly deduced from Figure 4A-D because the data presented are based on the per-protocol population, the CIs are one-sided and Bonferroni correction for three multiple comparisons is performed. The 2-4-6 schedule is non-inferior to the Dutch schedule for all serotypes and at all time-points, except for serotype 19A after the booster dose where the CI just crossed the non-inferiority margin of 0.5 (GMC ratio: 0.71; 95% CI: 0.49-1.03). The 3-5 schedule is non-inferior to the Dutch schedule for all serotypes and at all time-points, except for serotype 6B, 19F and 23F after the primary series. In addition, here the CI just crossed the non-inferiority margin of 0.5 (serotype 6B: GMC ratio: 0.73; 95% CI: 0.46-1.16; serotype 19F: GMC ratio: 0.74; 95% CI: 0.49-1.11; serotype 23F: GMC ratio: 0.71; 95% CI: 0.47-1.08). The 2-4 schedule is not non-inferior to the Dutch schedule for several serotypes at the time-points before the booster dose (serotype 3, 6B, 9V, 19A, 19F and 23F after the primary series, serotype 6B and 19F at 8 and 11 months), but is non-inferior to the Dutch schedule after the booster dose for all serotypes.

3.2.3 *Longitudinal analyses*

Figure 5 presents the longitudinal course of the GMC between the primary series and the booster dose, separately for the vaccination schedules and per pneumococcal serotype. Waning of antibodies was evident for all serotypes, although a significantly larger decline was seen in the first interval (from primary series to 8 months of age) compared with the second interval (from 8 months of age to booster dose), except for serotype 6B and 14. In general, the 2-4-6 and 3-5 schedules started with higher GMCs than the 2-3-4 and 2-4 schedules after the primary series. Thereafter the 2-4-6 and 3-5 schedules decreased more rapidly than the 2-3-4 and 2-4 schedules but the GMCs remained higher until the booster dose.

3.3 **Immunogenicity of co-administered DTaP-IPV-Hib vaccine**

In all four schemes, DTaP-IPV-Hib was given at 2,3,4 and 11 months of age. No significant differences between the four schedules were found for GMC/GMTs and seroprotection/seropositivity for DTaP-IPV-Hib components, except for polio type 2 (3-5 schedule superior to 2-3-4 schedule) and diphtheria (2-4-6 schedule superior to 2-3-4 and 2-4 schedule) before the booster dose (Table 3A and 3B). After the booster dose, the seroprotection against diphtheria and tetanus was 100% for all vaccination schedules. Seropositivity and seroprotection against pertussis toxin and *H. influenzae* b polysaccharide was 94% or higher for all vaccination schedules. Seroprotection against polio type 2 and 3 was high (ranging from 97-100%), except for schedule 2-3-4 (91%). Seroprotection against polio type 1 was somewhat lower (ranging from 90-94%).

Table 3A. Immunogenicity of routine vaccine (DTPa-IPV-Hib) administered at 2, 3, and 4 months of age and given concomitantly with or without PCV-13; GMC/GMTs and seroprotection/seropositivity rates measured before the booster dose

Vaccine Antigen	Threshold	2-4-6 schedule (n=94)		3-5 schedule (n=87)		2-3-4 schedule (n=86)		2-4 schedule (n=88)	
		GMC/GMT (95% CI)	%> Treshold	GMC/GMT (95% CI)	%> Treshold	GMC/GMT (95% CI)	%> Treshold	GMC/GMT (95% CI)	%> Treshold
Diphtheria	IgG ≥ 0•10 IU/ml	0•11 (0•09-0•13) ^{b,c}	57 ^{b,c}	0•08 (0•07-0•10)	46	0•07 (0•06-0•09)	37	0•07 (0•06-0•08)	36
Tetanus	IgG ≥ 0•10 IU/ml	0•20 (0•16-0•24)	78	0•19 (0•16-0•24)	69	0•15 (0•13-0•19)	66	0•18 (0•15-0•22)	69
Pertussis PT	IgG ≥ 20 EU/ml	8 (7-9)	7	9 (8-10)	7	8 (6-9)	10	8 (7-9)	5
Pertussis FHA	-	17 (15-20)	-	20 (16-24)	-	16 (13-19)	-	19 (16-24)	-
Pertussis PRN	-	7 (5-8)	-	9 (7-11)	-	6 (5-8)	-	6 (5-8)	-
Polio Type 1	Titers ≥ 1:8	5 (4-7)	37	6 (4-7)	40	5 (4-6)	38	6 (4-7)	42
Polio Type 2	Titers ≥ 1:8	9 (7-13)	49	16 (11-23) ^d	72 ^a	8 (6-11)	53	11 (8-15)	59
Polio Type 3	Titers ≥ 1:8	8 (6-11)	53	9 (6-13)	47	7 (5-10)	44	10 (7-14)	56
PRP-Hib	IgG ≥ 0•15 ug/ml	0•19 (0•14-0•27)	59	0•34 (0•24-0•49)	65	0•21 (0•15-0•30)	63	0•22 (0•16-0•31)	55

Abbreviations: GMC, geometric mean concentration; GMT, geometric mean titer; DTPa-IPV-Hib (Pediace^l[™], Sanofi Pasteur MSD, Inc). N indicates maximum number of infants with available results; actual number of infants included in the analysis varies slightly per antigen, depending on serum availability for testing. Differences between groups were tested using ANOVA and Chi-square or Fisher's Exact test where appropriate. P-value < 0•05 after adjustment for six multiple comparisons: (^a) 2-4-6 vs. 3-5; (^b) 2-4-6 vs. 2-3-4; (^c) 2-4-6 vs. 2-4; (^d) 3-5 vs. 2-3-4.

Table 3B. Immunogenicity of routine vaccine (DTPa-IPV-Hib) administered at 2, 3, 4 and 11-12 months of age and given concomitantly with or without PCV-13; GMC/GMTs and seroprotection/seropositivity rates measured one month after the booster dose

Vaccine Antigen	Threshold	2-4-6 schedule (n=92)		3-5 schedule (n=89)		2-3-4 schedule (n=86)		2-4 schedule (n=88)	
		GMC/GMT (95% CI)	%> Treshold	GMC/GMT (95% CI)	%> Treshold	GMC/GMT (95% CI)	%> Treshold	GMC/GMT (95% CI)	%> Treshold
Diphtheria	IgG ≥ 0•10 IU/ml	1•61 (1•39-1•86)	100	1•77 (1•51-2•06)	100	1•43 (1•25-1•63)	100	1•55 (1•38-1•74)	100
Tetanus	IgG ≥ 0•10 IU/ml	3•06 (2•55-3•67)	100	3•02 (2•51-3•62)	100	2•66 (2•24-3•15)	100	2•92 (2•44-3•48)	100
Pertussis PT	IgG ≥ 20 EU/ml	113 (95-135)	98	132 (112-155)	100	104 (87-124)	95	108 (93-127)	99
Pertussis FHA	-	82 (71-95)	-	96 (83-111)	-	83 (71-98)	-	91 (77-107)	-
Pertussis PRN	-	113 (93-137)	-	139 (112-174)	-	100 (83-120)	-	109 (89-134)	-
Polio Type 1	Titers ≥ 1:8	431 (278-670)	93	439 (285-678)	93	233 (143-378)	90	344 (226-524)	94
Polio Type 2	Titers ≥ 1:8	1169 (829-1649)	100	1294 (930-1799)	99	740 (506-1082)	100	1109 (787-1563)	98
Polio Type 3	Titers ≥ 1:8	1124 (750-1685)	99	1083 (704-1667)	98	651 (394-1074)	91	1386 (924-2080)	97
PRP-Hib	IgG ≥ 0•15 ug/ml	11•56 (8•05-16•59)	96	15•15 (10•38-22•11)	97	11•47 (7•64-17•22)	95	15•03 (11•21-20•14)	100

Abbreviations: GMC, geometric mean concentration; GMT, geometric mean titer; DTPa-IPV-Hib, Pediacel™, Sanofi Pasteur MSD, Inc. N indicates maximum number of infants with available results; actual number of infants included in the analysis varies slightly per antigen, depending on serum availability for testing. No significant differences between groups were found after testing adjusted for six multiple comparisons using ANOVA and Chi-square or Fisher's Exact test where appropriate.

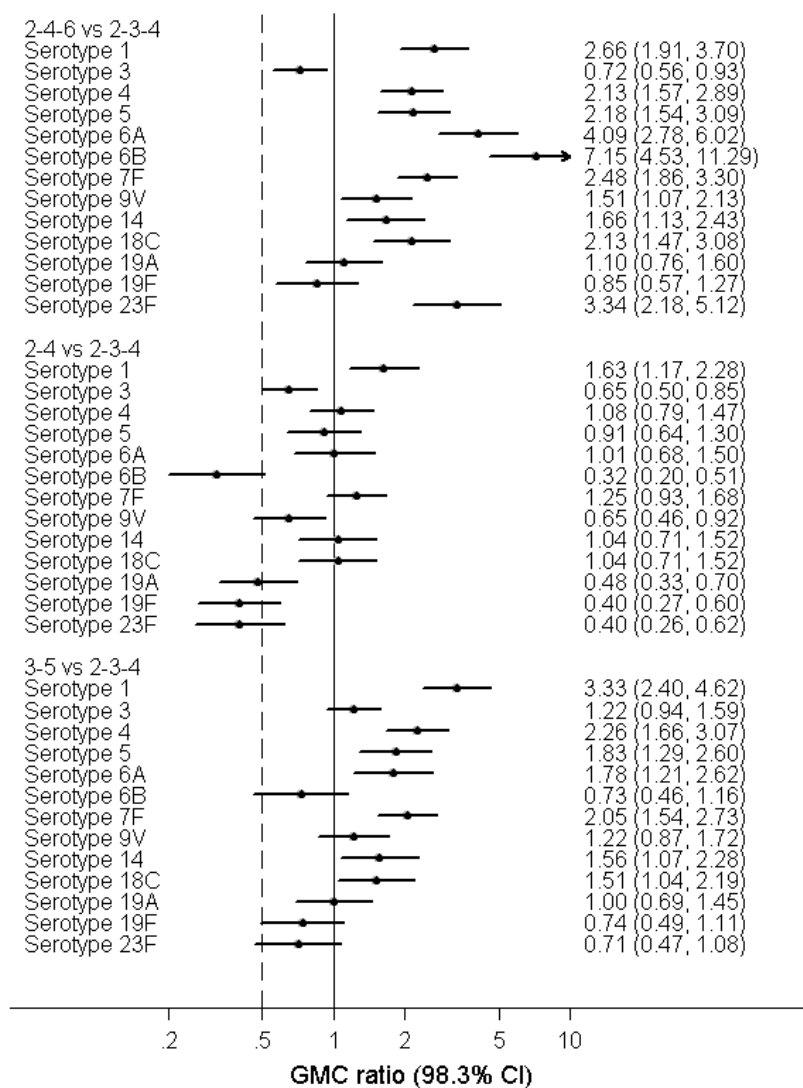


Figure 4A. Pneumococcal serotype-specific antibody GMC ratios with one-sided 98.3% confidence intervals (adjusted for three multiple comparisons) measured one month after the primary series in per-protocol population with the Dutch schedule as the reference.

Dashed line represents non-inferiority margin of 0.5. Note that superiority cannot be exactly deduced from this figure because the data presented is based on the per-protocol population, the CIs are one-sided and Bonferroni correction for three multiple comparisons is performed.

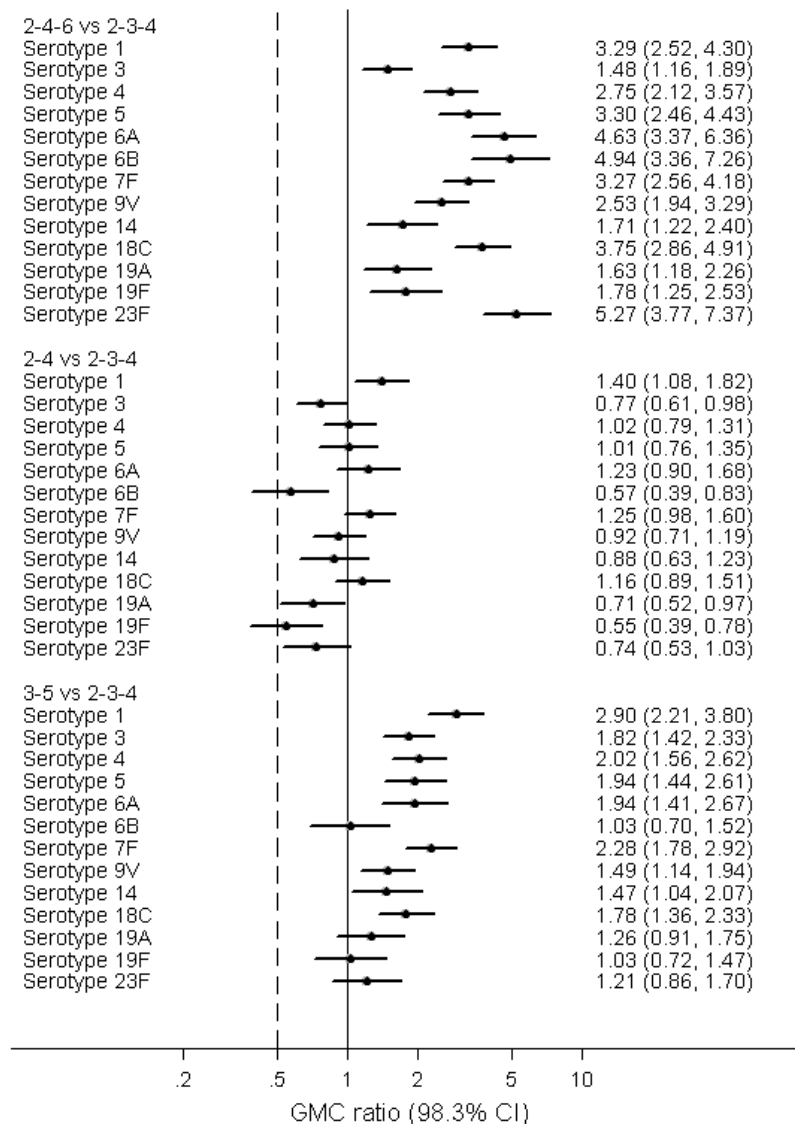


Figure 4B. Pneumococcal serotype-specific antibody GMC ratios with one-sided 98.3% confidence intervals (adjusted for three multiple comparisons) measured at 8 months of age in per-protocol population with the Dutch schedule as the reference.

Dashed line represents non-inferiority margin of 0.5. Note that superiority cannot be exactly deduced from this figure because the data presented is based on the per-protocol population, the CIs are one-sided and Bonferroni correction for three multiple comparisons is performed.

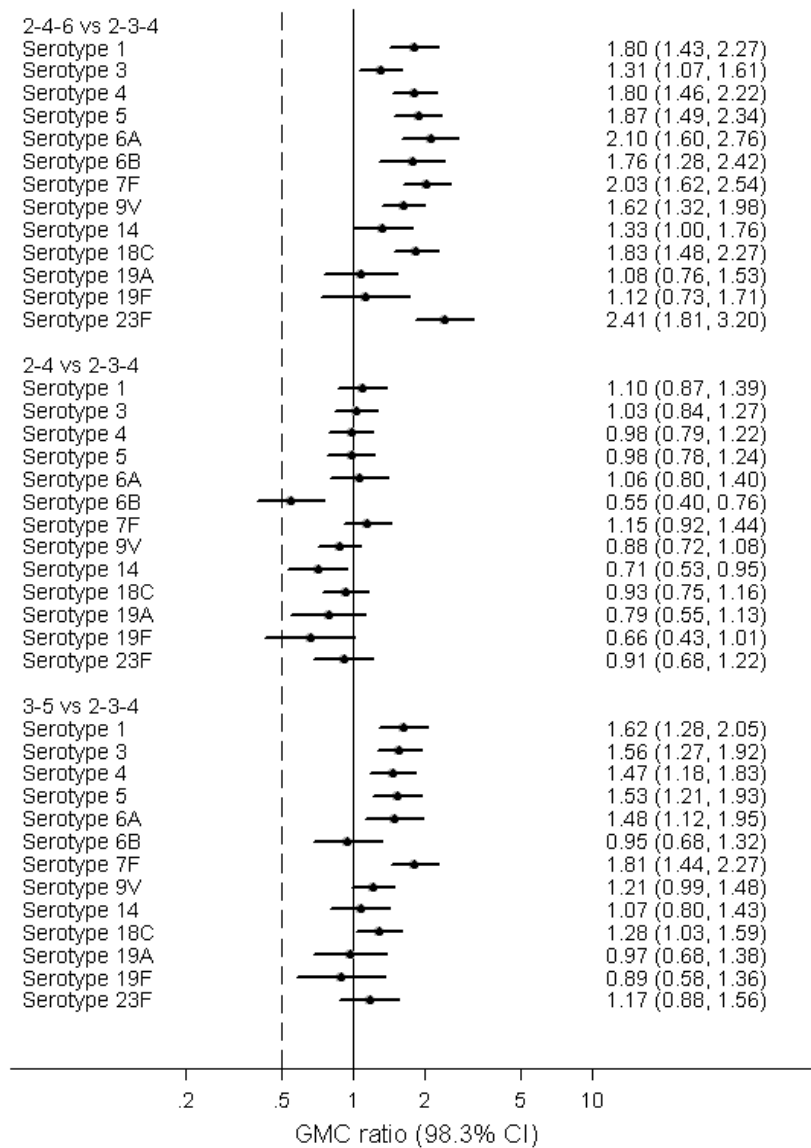


Figure 4C. Pneumococcal serotype-specific antibody GMC ratios with one-sided 98.3% confidence intervals (adjusted for three multiple comparisons) measured before the booster dose in per-protocol population with the Dutch schedule as the reference.

Dashed line represents non-inferiority margin of 0.5. Note that superiority cannot be exactly deduced from this figure because the data presented is based on the per-protocol population, the CIs are one-sided and Bonferroni correction for three multiple comparisons is performed.

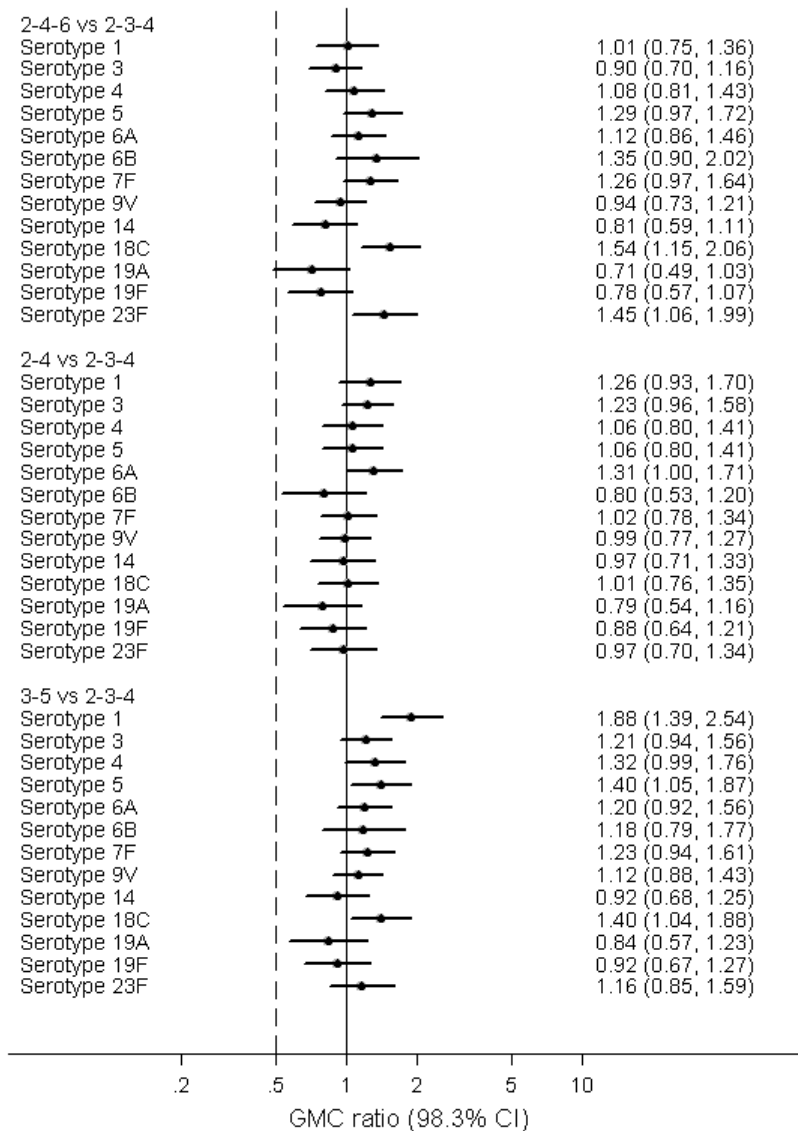


Figure 4D. Pneumococcal serotype-specific antibody GMC ratios with one-sided 98.3% confidence intervals (adjusted for three multiple comparisons) measured one month after the booster dose in per-protocol population with the Dutch schedule as the reference

Dashed line represents non-inferiority margin of 0.5. Note that superiority cannot be exactly deduced from this figure because the data presented is based on the per-protocol population, the CIs are one-sided and Bonferroni correction for three multiple comparisons is performed.

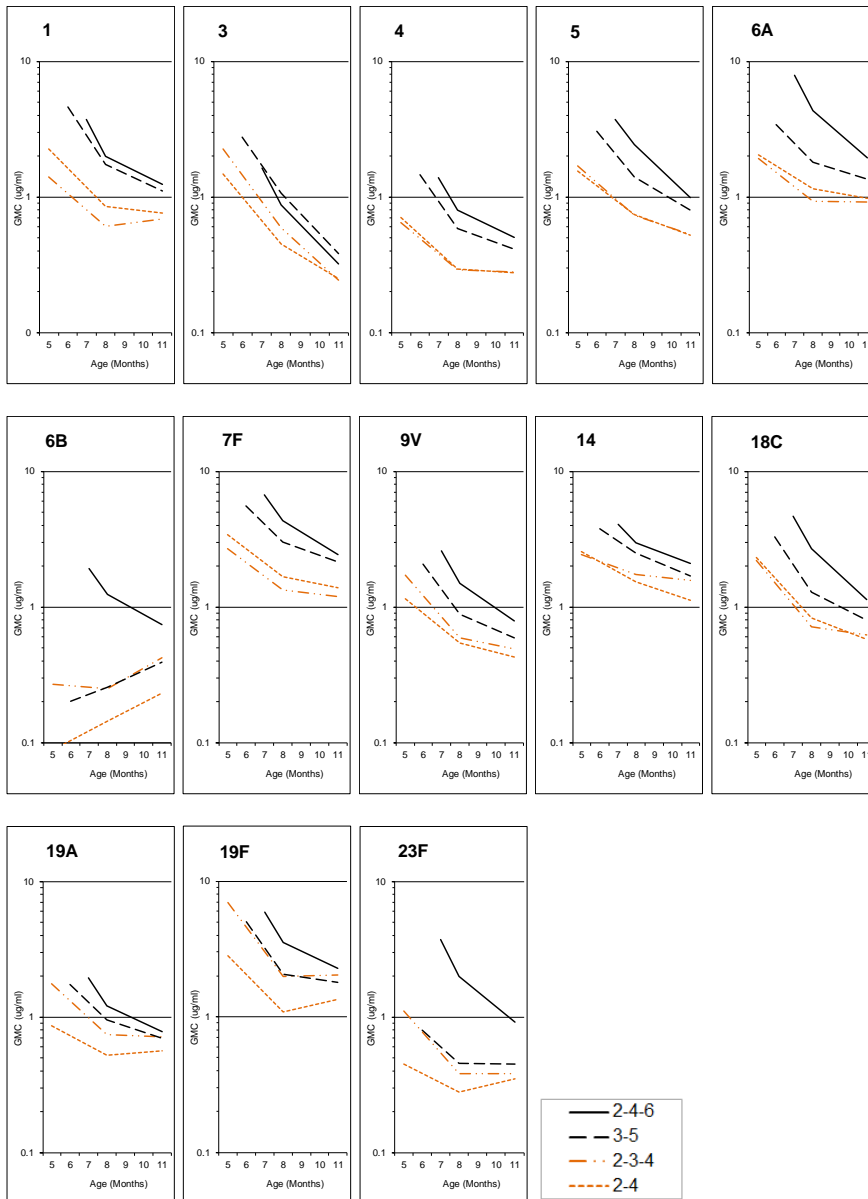


Figure 5. Longitudinal course of GMCs between the primary series and the booster dose per immunisation schedule per serotype

3.4 Supplemental data about carriage and IPD in the Netherlands

Information about IPD incidence and serotype-specific carriage is essential to be able to interpret the above findings. We assessed the number of IPD cases based on data collected the Netherlands Reference Laboratory for Bacterial Meningitis from 9 sentinel medical microbiology laboratories that represent 25% of the Dutch population. Carriage data were obtained from several carriage studies performed in the Netherlands (12, 13).

Figures 6A and 6B show IPD incidence before and after implementation of PCV7 in the NIP in 0 to 4-year-old children and in persons older than 4 years. Obviously, IPD caused by vaccine serotypes decreased in young age children but also already 2-3 years after PCV7 implementation in the population older than 4 years due to herd immunity. However, particularly in the population older than 4 years serotype replacement caused an increase of IPD by non-vaccine serotypes nullifying the herd immunity effects for the vaccine serotypes. Table 4 summarizes the Dutch data about the number of IPD cases and prevalence of carriage before and after implementation of PCV7.

Table 4. Number of IPD cases and prevalence of carriage and in the Netherlands for serotypes included in PCV13

Serotype	Number of IPD cases in nine sentinel labs representing 25% of the Dutch population				Carriage in 11-month old children (%)		
	2004-2005	2007-2008	2009-2010	2011*	May – Dec 2006	Feb – July 2009	Sept 2010 – Jan 2011
1	94	142	122	42	< 1.5	< 1.5	< 1.5
3	73	65	81	38	< 1.5	< 1.5	< 1.5
4	107	86	50	29	0	0	0
5	8	5	13	11	< 1.5	< 1.5	< 1.5
6A	36	30	22	3	6	3	1
6B	40	55	21	3	8	4	1
7F	140	137	168	102	< 1.5	< 1.5	< 1.5
9V	85	101	48	7	3	0	0
14	188	145	55	20	3	0	0
18C	29	35	24	10	2	0	0
19A	42	59	92	72	2	10	12
19F	40	24	19	9	11	2	1
23F	73	61	29	7	11	2	1

*Note that this is only one year, so number of IPD cases are smaller

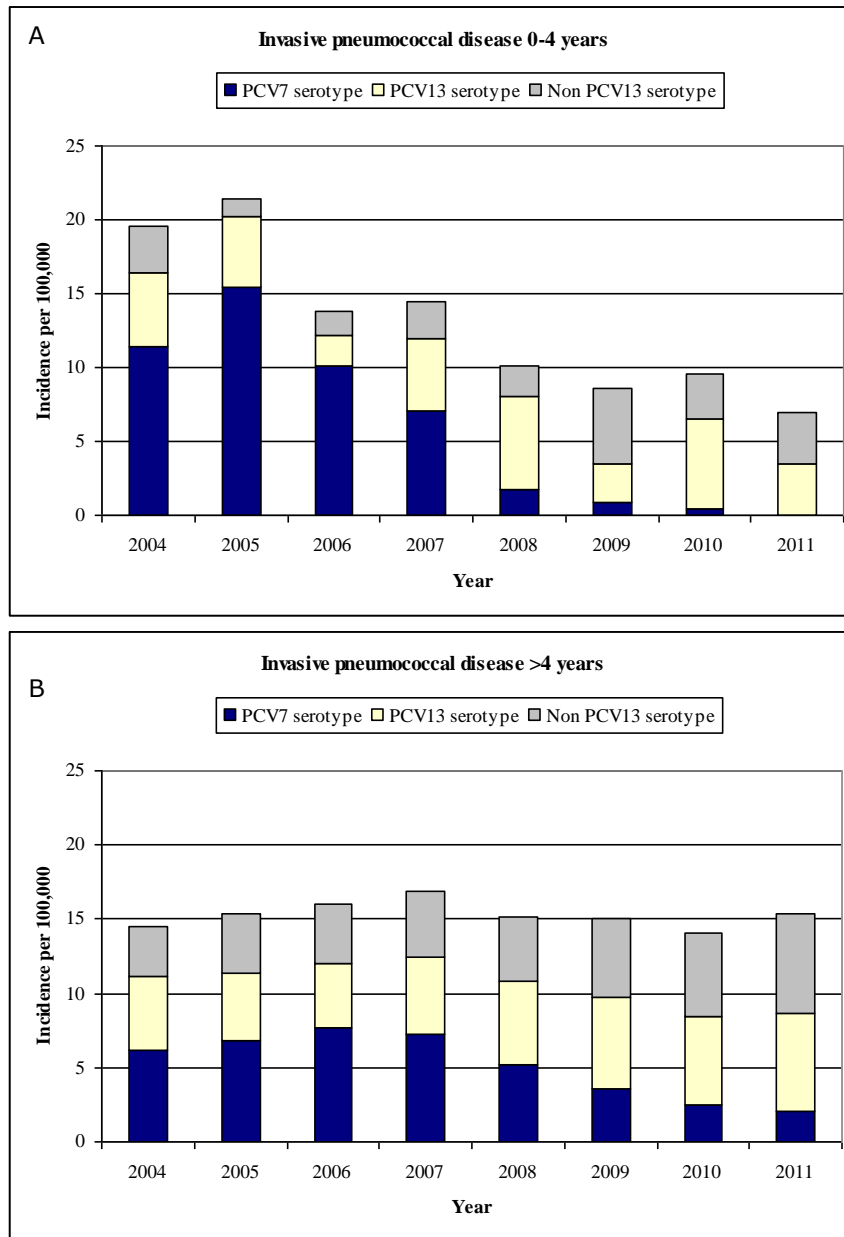


Figure 6. IPD incidence by PCV7 serotypes and non-PCV7 serotypes before and after implementation of PCV7 in 0 to 4-year-old children (A) and persons older than 4 years (B)

Nationwide vaccination with PCV7 without a catch-up campaign was implemented in the Netherlands in 2006 and replaced by vaccination with PCV10 in 2010.

4 Discussion

4.1 Summary of findings

After the booster dose at 12 months, virtually no differences in IgG antibody levels against the 13 vaccine serotypes existed between the schedules and the seroprotection (antibody concentration > 0.35 µg/ml) was almost 100% for all serotypes for all schedules. However, when assessing the period between the primary series and the booster dose, which is the time interval most IPD cases in children occur, some schedules yielded higher IgG concentrations than others. The 2-4-6 schedule was clearly superior to the Dutch schedule for many serotypes before the booster dose, but includes three primary doses. The 3-5 schedule, which has the advantage of a reduced number of doses, was also superior to the Dutch schedule for the serotypes 1, 3, 4, 5, 6A, 7F, 9V and 18C, but was just crossing the non-inferiority border for serotype 6B, 19F and 23F after the primary series. The 2-4 schedule was clearly inferior to the Dutch schedule for several serotypes. At 8 months of age, seroprotection was above 80% (> 0.35 µg/ml) for all serotypes in the 2-4-6 schedule and for almost all serotypes in the 3-5 schedule (serotype 4: 74%, serotype 6B: 39%). At the same time point, seroprotection was less than 80% for serotype 1, 3, 4, 6B, 9V, 19F and 23F in the 2-3-4 schedule and for serotype 3, 4, 6B, 9V, 19A and 23F in the 2-4 schedule.

4.2 Comparison with other immunogenicity studies

Comparative studies on the required number of vaccine doses and the optimal timing for the primary series and subsequent booster vaccination are scarce. In a study of Goldblatt et al. (14) an experimental 9-valent pneumococcal conjugate vaccine from Wyeth was administered in a 3+1 schedule (2-3-4-12 months) and in a 2+1 schedule (2-4-12). No significant differences between the results obtained by the 2+1 and 3+1 schedules were found. However, there are a number of caveats and flaws to this study. Firstly, the vaccine used was not PCV7 or PCV13, but a 9-valent predecessor of PCV7. The 9-valent vaccine had a higher immunogenicity than Prevenar-7, which might have influenced responses. Secondly, the group of children enrolled in the 2+1 and 3+1 schedules were recruited during two different periods (2000-2001 and 2001-2003, respectively) and originated from two different geographic locations. Furthermore, the groups of children that were used for comparative serology were small: 24 children for the 3+1 group and 36 children for the 2+1 group. These numbers may be too small to be able to detect a significant 2-fold difference in antibody concentrations between schedules. The study also included groups of children receiving a booster with the 23-valent polysaccharide vaccine. In 2008 Sigurdardottir et al. described a vaccination study with a 9-valent pneumococcal conjugate vaccine in a comparative study using a 2+1 and a 3+1 dose schedule (15). They showed that antibody concentrations were higher in the 3+1 schedule. This study was also not performed with the currently licensed vaccines and part of the children received a booster vaccination with a non-conjugated pneumococcal polysaccharide vaccine. The MINOES study (ISRCTN25571720) by Sanders et al. also compared different reduced schedules on carriage and antibody responses (13). Children receiving the 2+1 schedule in that study had pre- and postbooster GMC levels of anti-PS IgG comparable with the regular 3+1 schedule, as measured in the aKwK study (ISRCTN97785537) by Berbers et al., with the exception of serotypes 6B and 23F (16). Although the MINOES study yielded very valuable

data on reduced dose schedules showing a similar reduction in carriage of vaccine serotypes, it cannot be considered as a direct comparison between 4 and 3 doses PCV7 vaccination schedules with respect to IgG antibody levels. Recently Dagan et al. compared a 3+1 vaccination schedule with a 2+1 schedule using PCV7 and obtained results that were similar to those from the PIM-study with reduced antibody concentrations against serotypes 6B and 23F (17). They also observed a higher acquisition and prevalence of these serotypes in the 2+1 vaccinated children before the booster dose was given. After the booster, no differences in carriage between the 3+1 and 2+1 groups were observed. None of the comparative studies directly compared four different schedules and all were performed with PCV7 or PCV9 and not with PCV13. In addition, these studies did not investigate the period between 4 and 11 months of age in which incidence of pneumococcal infection peaks and the decline in antibody concentrations is not clear. Therefore, a direct comparison with the PIM study results is seriously hampered.

4.3 Effectiveness of reduced dose schedule

There are as yet no studies available that directly compared 2+1 and 3+1 dose schedules on IPD outcomes with the 7 or 13-valent vaccine. Very recent results of a study in Finland with a 10-valent vaccine administered in a 3+1 schedule showed no vaccine failures and up to 100% protection against vaccine serotypes IPD vs 92% in a 2+1 schedule, though IPD cases were few (ISPPD and ESPID 2012, abstract). Previously, a case-control study in the US showed effectiveness against vaccine serotype IPD of 98% (95% CI: 75-100%) for a 2+1 dose schedule (18). Furthermore, in this study no significant difference was found on risk of IPD for children vaccinated with a 2+1 or 2+0 dose schedule as compared with a 3+0 dose schedule, although the number of cases and therefore power was very limited to make comparisons and protection by herd immunity cannot be excluded. In 11 European countries a 2+1 dose schedule, and specifically a 3-5-12 schedule is implemented for pneumococcal vaccination in Norway, Sweden, Finland and Denmark. No effectiveness data from Sweden and Finland are available, but observational studies from Denmark and Norway show high vaccine effectiveness (19, 20). Denmark introduced PCV7 in October 2007 in their NIP at the ages of 3, 5 and 12 months with a catch-up programme for children up to 17 months. Surveillance data from Denmark before (2000-2007) and after (2008-2010) implementation of PCV7 vaccination showed that the incidence of vaccine type IPD decreased with 89% (95% CI: 82-94%) in children below 2 years of age. PCV7 was introduced in the Norwegian NIP in July 2006 also at ages of 3, 5 and 12 months, with a catch-up for all children born in 2006. Vaccine type IPD incidence decreased with 71% (95% CI: 50-83%) from 2004-2005 to 2007 in children below 2 years of age. The vaccine effectiveness in Denmark and Norway is comparable to that in the Netherlands, where a 67% (95% CI: 41-81%) decrease was observed in children below 2 years of age when comparing vaccine type IPD incidence between 2004-2006 and 2006-2008 (21).

Several cost-effectiveness studies compared 2+1 dose schedules with 3+1 dose schedules. Rozenbaum *et al.* showed that a 2+1 dose schedule would be more cost-effective than a 3+1 dose schedule assuming similar effectiveness on IPD of a reduced dose schedule (22). With 13-valent PCV and inclusion of indirect effects (herd immunity and serotype replacement), the incremental cost effectiveness ratio would be 50,042 euros per QALY for a 3+1 dose schedule and 35,743 euros per QALY for a 2+1 dose schedule. Also other studies in Norway and Germany found increased cost-effectiveness of 2+1 versus 3+1 dose schedules (23, 24).

4.4 Possible effects of reduced vaccination schedule

The 3-5 vaccination schedule was superior to the Dutch vaccination schedule with regard to serotypes 1, 3, 4, 5, 6A, 7F, 9V and 18C. Serotype 1 and 7F are currently the serotypes causing most IPD. In addition, serotype 3 and 9V often cause IPD. A better protection against these serotypes will be achieved with the 3-5 schedule and this may reduce the number of IPD cases further. The 3-5 schedule was marginally not non-inferior to the Dutch schedule with regard to serotypes 6B, 19F and 23F but only 1 month after the primary series. Carriage of these three serotypes was substantial before introduction of PCV7, but reduced to 1% after implementation of PCV7. In addition, the number of IPD cases due to these serotypes reduced substantially after introduction of PCV7. Therefore, slightly less protection against these three serotypes with the 3-5 schedule as compared with the 2-3-4 schedule will probably not have a noticeable impact on IPD. However, although vaccine failure after pneumococcal vaccination is very rare in the Netherlands, the few cases of vaccine failure that have occurred involved serotypes 6B, 18C and 19F only in the first years after implementation of PCV7.

Due to the nationwide vaccination with PCV7 and recently with PCV10, considerable herd immunity has been mounted which has nearly eliminated circulation of the vaccine serotypes. This will most likely prevent any negative effects of the reduced dose 3-5 vaccination schedule caused by such as the delayed start and the larger between dose intervals of this schedule. IPD cases occurring before the age of 3 months cannot be prevented by the 3-5 schedule but these children will be protected by herd effects once vaccine serotypes do not circulate anymore (25). Non-vaccine serotype IPD cannot be prevented by vaccination.

4.5 Strengths and limitations of the study

This is the first study that compared four internationally used vaccination schedules for pneumococcal vaccines in the first year of life within the same region of the same country. By keeping all the circumstances for all participants the same within the study, a direct comparison between the immunogenicity profiles induced by these four schedules is reliable. The immune responses were measured at 4 different time points in samples from the same children providing a longitudinally comparison of the kinetics of the antibody levels.

In this study, the DTaP-IPV-Hib vaccine was administered at 2, 3, 4 and 11 months of age in all randomization groups, as is current practice in the Netherlands. This means that the number of co-administrations of PCV13 and DTaP-IPV-Hib vaccine differed between the randomization groups, e.g. children in the 2-3-4 schedule group received the two vaccines simultaneously at all three moments, while children in the 3-5 schedule group only received the two vaccines simultaneously at one moment. This difference in co-administration could have influenced the antibody response to PCV13. However, a previous study showed that antibody responses against pneumococcal serotypes were similar after PCV7, irrespective of concurrent or sequential administration of DTaP-IPV-Hib and Hepatitis B vaccines (26).

4.6 Seroprotection

Serum antibody concentrations were used as correlates of protection against invasive pneumococcal disease. Generally, good correlation has been demonstrated for all PCV13 serotypes between the quantity of the immune response, i.e. antibody titres, and the quality of the immune response, i.e.

opsonophagocytic activity (27). Data from three randomised controlled trials were pooled to derive a protective antibody concentration of 0.35 µg/ml for all PCV-7 serotypes combined when using standard ELISA (28). However, use of this threshold for all serotypes might be inappropriate, being too high for some (e.g. serotype 6B) and too low for others (e.g. serotype 19F) (6). Furthermore, no threshold is known for the six additional serotypes included in PCV-13 due to lack of efficacy data. Therefore, seroprotection levels should be interpreted with caution.

Although the correlation between the standard WHO-ELISA and the MIA as used in this study is high, the MIA tend to result in somewhat higher antibody values than the standard ELISA for some serotypes (8). This does not influence the conclusions drawn on the comparison of the four vaccination schedules in this study, because the schedules are compared mutually within the study but absolute percentages of seroprotection should be interpreted with caution. In addition, in a comparison with other studies this should be taken into account.

4.7 Interference and implementation

A switch of vaccination schedule for pneumococcal vaccination should be seen in light of the optimisation of vaccination schedule of vaccines administered concomitantly in the Dutch NIP. It is unlikely that a switch from 2-3-4 to a 3-5 or 2-4-6 schedule for DTaP-IPV-Hib-HepB vaccination will lead to less protection against diphtheria, tetanus, polio, *H. influenzae* b disease or hepatitis B, although future research to corroborate this assumption may be needed. A switch to a 3-5 schedule may have consequences for the protection against pertussis, a disease that may be accompanied by serious clinical manifestations especially in the first months after birth. A thorough evaluation of the implications of delaying pertussis vaccination with one month should be performed, and alternative solutions for protection against pertussis should be explored, such as maternal vaccination, cocooning and isolated pertussis vaccination in the first months after birth. For protection against pertussis, there is evidence that a single dose already prevents the development of (severe) disease (29-31) so delay of a first a single dose of conjugate vaccine may have some implications.

In the current health care system, visits at the well baby clinics are planned at 1, 2, 3, 4 and 6 months in the first half year of life. Although no visit is planned at 5 months the vaccination visit at 4 months could be postponed to 5 months or the visits of 4 and 6 months could be combined at 5 months with the administration of the last vaccination from the primary series and a physical check of the baby. Therefore, implementation of the 3-5 schedule seems feasible using a 2-3-5 schedule for all vaccinations of the primary series of the NIP. At 2 months, only the DTaP-IPV-Hib-HepB combination vaccine is given and at 3 and 5 months, the PCV10 and DTaP-IPV-Hib-HepB vaccines are simultaneously administered. Thus, implementation of the reduced 3-5-11 schedule for PCV vaccination is feasible in the NIP with a minimal change in the visit schedule.

5 Conclusions

In conclusion, after the booster dose at 12 months of age all four immunisation schedules investigated in this study show adequate protection against IPD, and virtually no differences in induced IgG antibody levels between the four schedules existed. In the period between the primary series and the booster dose, the 2-4-6 and 3-5 schedule are clearly better than the 2-3-4 and 2-4 schedule. When opting for a reduced dose schedule, the 3-5 schedule is the best choice offering a high level of seroprotection. Clinical effectiveness of the 3-5 schedule is confirmed by countries that have already implemented this schedule in their NIP. In addition, the already existing herd immunity against the seven serotypes included in PCV7 and the three additional serotypes in PCV10 supports safe implementation of the 3-5 schedule. We therefore recommend the introduction of the reduced 3-5-11 pneumococcal vaccination schedule in the Dutch NIP for protection against IPD which might be easily implemented when a 2-3-5 schedule for the primary vaccinations of the other components in the NIP is introduced leaving the start of the pertussis vaccinations at 2 months.

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7 Supplementary tables and figures

Supplementary Table 1A. Summary table showing superiority, inferiority and non-inferiority of alternative schedules compared with the Dutch schedule for pneumococcal serotype-specific antibody GMCs measured one month after the primary series

Serotype	2-4-6 schedule vs 2-3-4 schedule			2-4 schedule vs 2-3-4 schedule			3-5 schedule vs 2-3-4 schedule		
	Superiority*	Inferiority*	Non-inferiority**	Superiority*	Inferiority*	Non-inferiority**	Superiority*	Inferiority*	Non-inferiority**
1	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes
3	No	Yes	Yes	No	Yes	No	No	No	Yes
4	Yes	No	Yes	No	No	Yes	Yes	No	Yes
5	Yes	No	Yes	No	No	Yes	Yes	No	Yes
6A	Yes	No	Yes	No	No	Yes	Yes	No	Yes
6B	Yes	No	Yes	No	Yes	No	No	No	No
7F	Yes	No	Yes	No	No	Yes	Yes	No	Yes
9V	No	No	Yes	No	No	No	No	No	Yes
14	Yes	No	Yes	No	No	Yes	No	No	Yes
18C	Yes	No	Yes	No	No	Yes	No	No	Yes
19A	No	No	Yes	No	Yes	No	No	No	Yes
19F	No	No	Yes	No	Yes	No	No	No	No
23F	Yes	No	Yes	No	Yes	No	No	No	No

* Based on intention-to-treat population, 2-sided tests, Bonferroni correction for six multiple comparisons

** Based on per-protocol population, 1-sided tests, Bonferroni correction for three multiple comparisons, NI margin is GMC ratio of 0.5

Supplementary Table 1B. Summary table showing superiority, inferiority and non-inferiority of alternative schedules compared with the Dutch schedule for pneumococcal serotype-specific antibody GMCs measured at 8 months of age

Serotype	2-4-6 schedule vs 2-3-4 schedule			2-4 schedule vs 2-3-4 schedule			3-5 schedule vs 2-3-4 schedule		
	Superiority*	Inferiority*	Non-inferiority**	Superiority*	Inferiority*	Non-inferiority**	Superiority*	Inferiority*	Non-inferiority**
1	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes
3	Yes	No	Yes	No	No	Yes	Yes	No	Yes
4	Yes	No	Yes	No	No	Yes	Yes	No	Yes
5	Yes	No	Yes	No	No	Yes	Yes	No	Yes
6A	Yes	No	Yes	No	No	Yes	Yes	No	Yes
6B	Yes	No	Yes	No	Yes	No	No	No	Yes
7F	Yes	No	Yes	No	No	Yes	Yes	No	Yes
9V	Yes	No	Yes	No	No	Yes	Yes	No	Yes
14	Yes	No	Yes	No	No	Yes	No	No	Yes
18C	Yes	No	Yes	No	No	Yes	Yes	No	Yes
19A	Yes	No	Yes	No	No	Yes	No	No	Yes
19F	Yes	No	Yes	No	Yes	No	No	No	Yes
23F	Yes	No	Yes	No	No	Yes	No	No	Yes

* Based on intention-to-treat population, 2-sided tests, Bonferroni correction for six multiple comparisons

** Based on per-protocol population, 1-sided tests, Bonferroni correction for three multiple comparisons, NI margin is GMC ratio of 0.5

Supplementary Table 1C. Summary table showing superiority, inferiority and non-inferiority of alternative schedules compared with the Dutch schedule for pneumococcal serotype-specific antibody GMCs measured before the booster dose

Serotype	2-4-6 schedule vs 2-3-4 schedule			2-4 schedule vs 2-3-4 schedule			3-5 schedule vs 2-3-4 schedule		
	Superiority*	Inferiority*	Non-inferiority**	Superiority*	Inferiority*	Non-inferiority**	Superiority*	Inferiority*	Non-inferiority**
1	Yes	No	Yes	No	No	Yes	Yes	No	Yes
3	Yes	No	Yes	No	No	Yes	Yes	No	Yes
4	Yes	No	Yes	No	No	Yes	Yes	No	Yes
5	Yes	No	Yes	No	No	Yes	Yes	No	Yes
6A	Yes	No	Yes	No	No	Yes	Yes	No	Yes
6B	Yes	No	Yes	No	Yes	No	No	No	Yes
7F	Yes	No	Yes	No	No	Yes	Yes	No	Yes
9V	Yes	No	Yes	No	No	Yes	No	No	Yes
14	No	No	Yes	No	No	Yes	No	No	Yes
18C	Yes	No	Yes	No	No	Yes	No	No	Yes
19A	No	No	Yes	No	No	Yes	No	No	Yes
19F	No	No	Yes	No	No	No	No	No	Yes
23F	Yes	No	Yes	No	No	Yes	No	No	Yes

* Based on intention-to-treat population, 2-sided tests, Bonferroni correction for six multiple comparisons

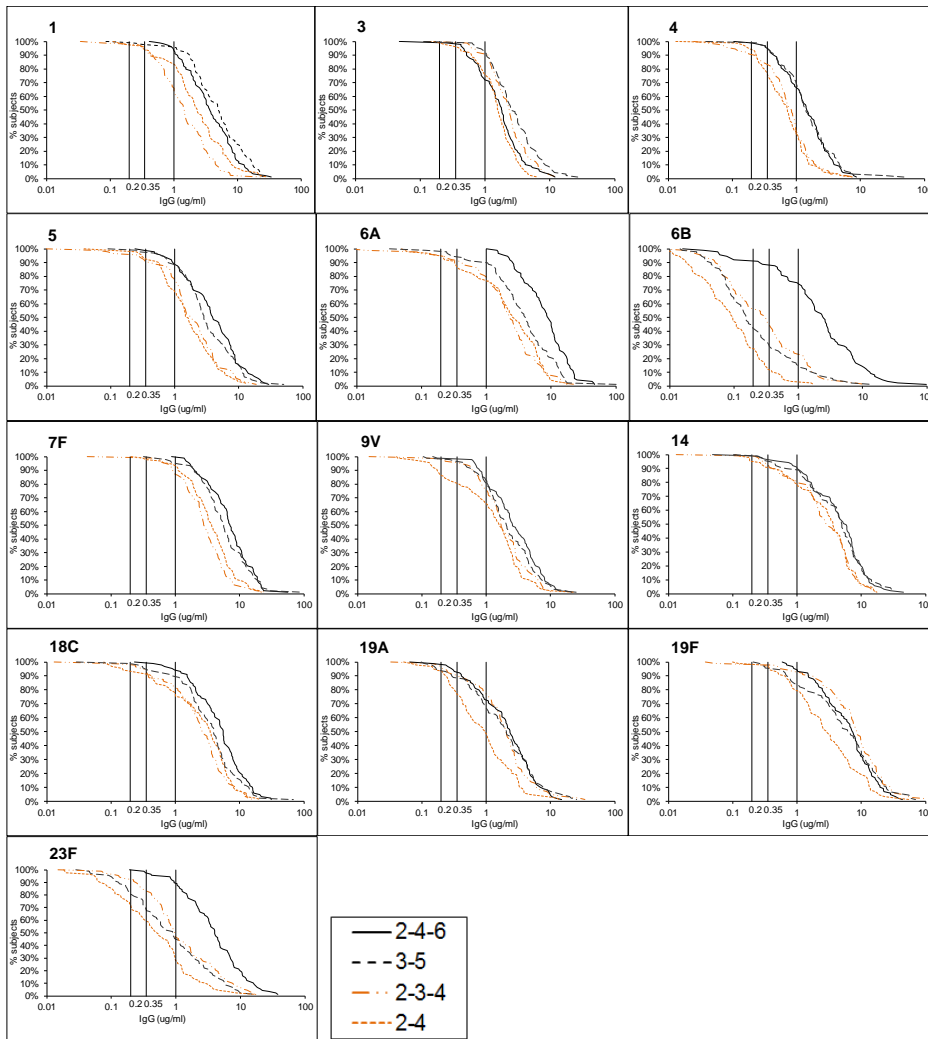
** Based on per-protocol population, 1-sided tests, Bonferroni correction for three multiple comparisons, NI margin is GMC ratio of 0.5

Supplementary Table 1D. Summary table showing superiority, inferiority and non-inferiority of alternative schedules compared with the Dutch schedule for pneumococcal serotype-specific antibody GMCs measured one month after the booster dose

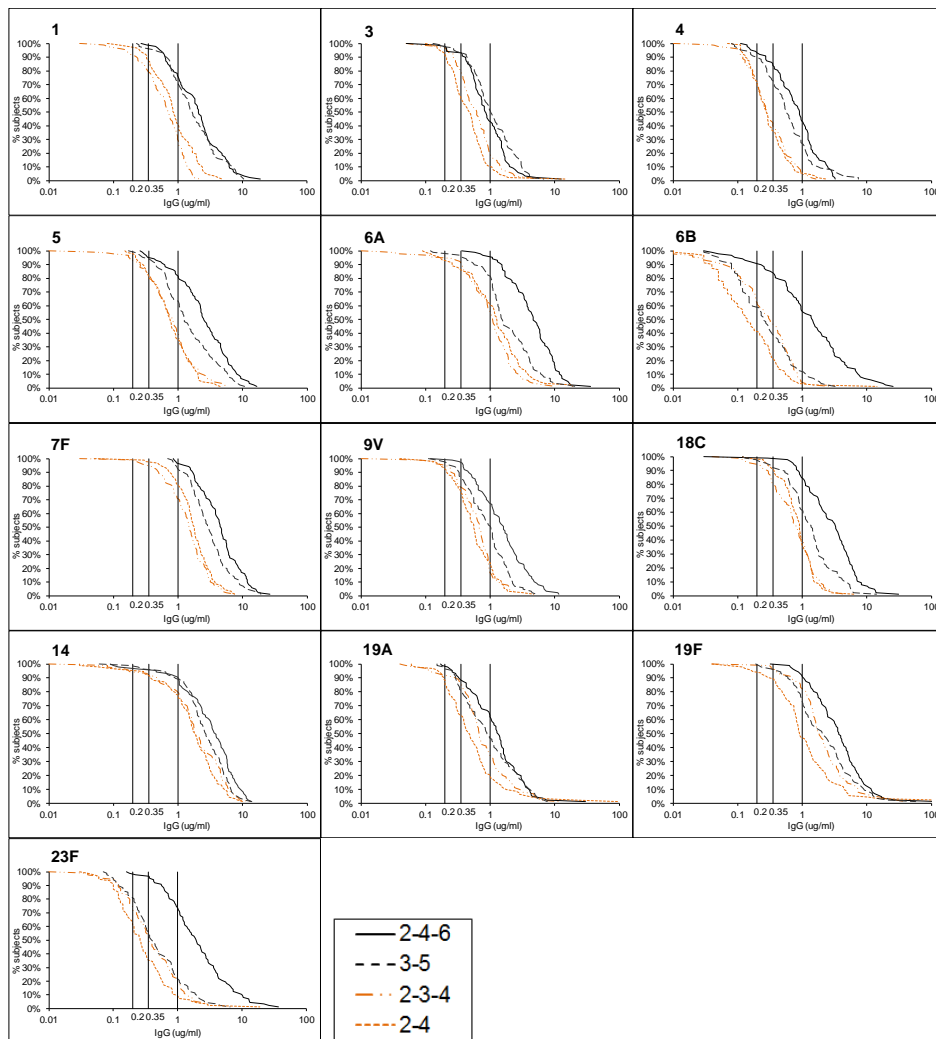
Serotype	2-4-6 schedule vs 2-3-4 schedule			2-4 schedule vs 2-3-4 schedule			3-5 schedule vs 2-3-4 schedule		
	Superiority*	Inferiority*	Non-inferiority**	Superiority*	Inferiority*	Non-inferiority**	Superiority*	Inferiority*	Non-inferiority**
1	No	No	Yes	No	No	Yes	Yes	No	Yes
3	No	No	Yes	No	No	Yes	No	No	Yes
4	No	No	Yes	No	No	Yes	No	No	Yes
5	No	No	Yes	No	No	Yes	No	No	Yes
6A	No	No	Yes	No	No	Yes	No	No	Yes
6B	No	No	Yes	No	No	Yes	No	No	Yes
7F	No	No	Yes	No	No	Yes	No	No	Yes
9V	No	No	Yes	No	No	Yes	No	No	Yes
14	No	No	Yes	No	No	Yes	No	No	Yes
18C	Yes	No	Yes	No	No	Yes	No	No	Yes
19A	No	No	No	No	No	Yes	No	No	Yes
19F	No	No	Yes	No	No	Yes	No	No	Yes
23F	Yes	No	Yes	No	No	Yes	No	No	Yes

* Based on intention-to-treat population, 2-sided tests, Bonferroni correction for six multiple comparisons

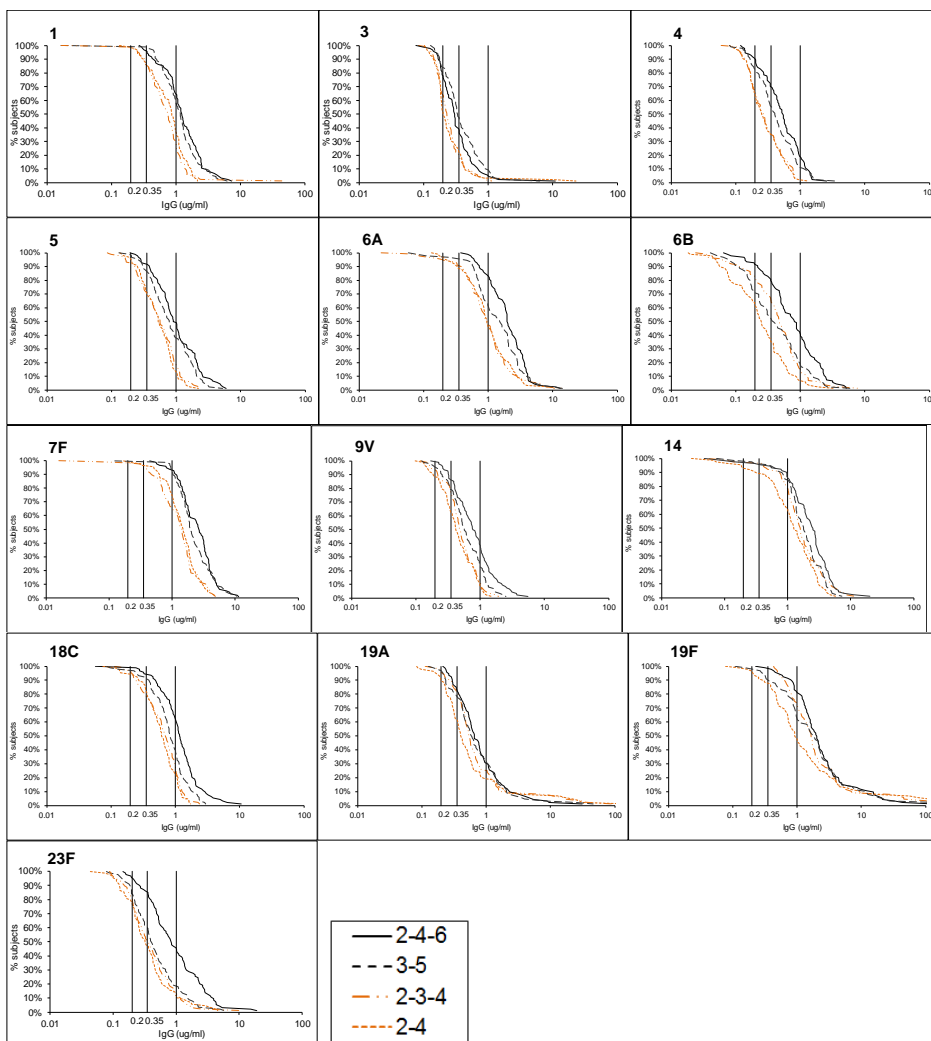
** Based on per-protocol population, 1-sided tests, Bonferroni correction for three multiple comparisons, NI margin is GMC ratio of 0.5



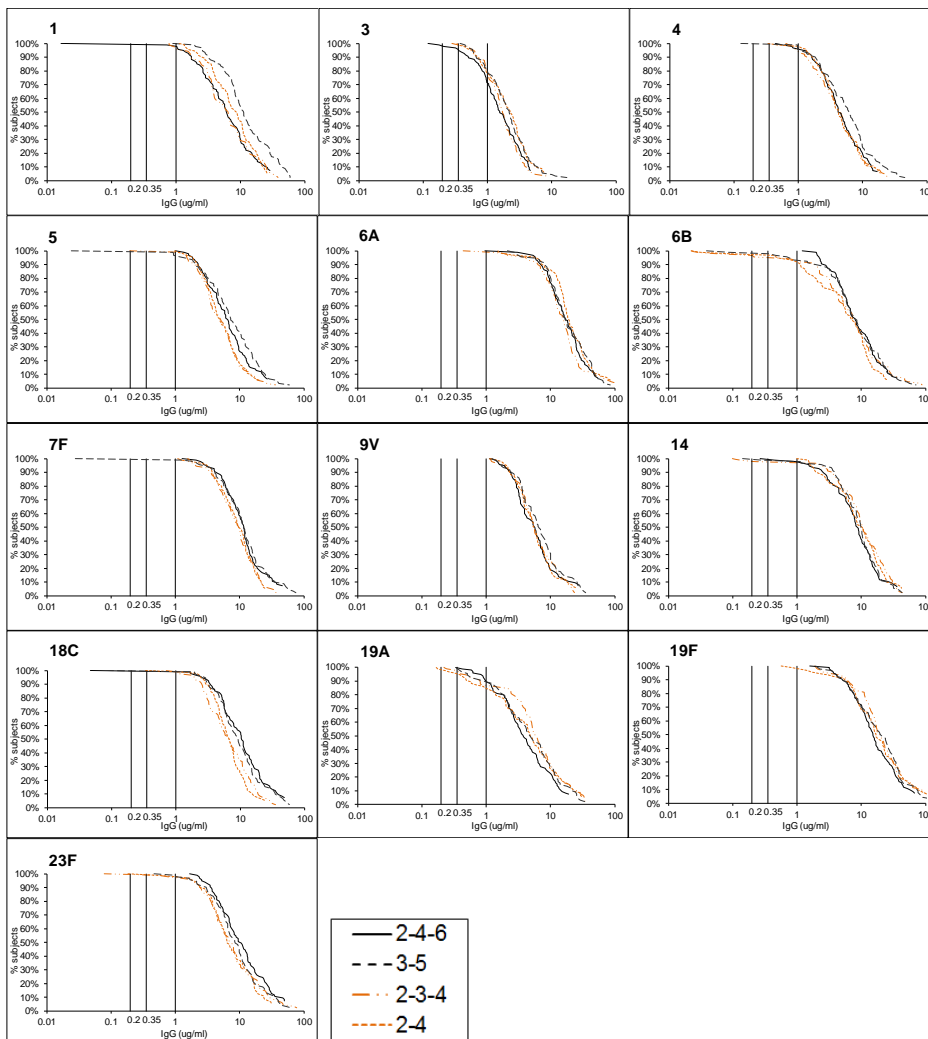
Supplementary figure 1A. Relative cumulative distribution curves of the IgG concentrations per immunisation schedule per serotype one month after the primary series



Supplementary figure 1B. Relative cumulative distribution curves of the IgG concentrations per immunisation schedule per serotype at 8 months of age



Supplementary figure 1C. Relative cumulative distribution curves of the IgG concentrations per immunisation schedule per serotype before the booster dose at 11 months of age



Supplementary figure 1D. Relative cumulative distribution curves of the IgG concentrations per immunisation schedule per serotype one month after the booster dose at 12 months of age

