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Comparability of antibody response to a booster dose of 7-valent pneumococcal conjugate vaccine in infants primed with either 2 or 3 doses

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ABSTRACT

In this cohort study we compared IgG antibody levels between infants immunized with 7-valent CRM197-conjugated pneumococcal vaccine (PCV-7) at 2, 4 and 11 months and at 2, 3, 4 and 11 months of age, as measured by double adsorption ELISA. Pre- and post-booster levels following the 2 + 1- and 3 + 1-dose schedule were comparable for 5 out of 7 serotypes except for serotypes 6B and 19F. The proportion of children reaching post-booster antibody thresholds were comparable except for 6B ($\geq 1.0 \mu\text{g/ml}$ and $\geq 5.0 \mu\text{g/ml}$) and 19F ($\geq 5.0 \mu\text{g/ml}$). Surveillance studies are warranted for vaccine impact on 6B and 19F disease cases after reduced-dose PCV-7 schedules.

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1. Introduction

Streptococcus pneumoniae is a leading cause of bacterial infections in children in the first years of life with clinical syndromes varying from non-invasive respiratory disease (pneumonia, otitis media) to invasive pneumococcal disease (IPD; sepsis, bacteremia and meningitis) [1]. In 2000, the CRM197-conjugated 7-valent pneumococcal vaccine (PCV-7) was licensed in the USA for infants for prevention of IPD and recommended in a 3 + 1 vaccination schedule with 3 primary doses in the first 6 months of life, followed by a booster dose in the second year of life. Clinically, protection against IPD after less than 4 doses was already observed in the licensure study for CRM197-conjugated PCV-7, the Northern California Kaiser Permanente study [2]. In this study, clinical efficacy against vaccine serotype IPD in the intention to treat analysis was high (93.9%) despite the fact that only 58% of the children had received the full PCV-7 schedule. Furthermore, protection by reduced-dose schedules in preventing vaccine serotype IPD in vaccinees was

observed in a large case-control study from the USA showing high effectiveness with a 2 + 1-dose (98%, 95% confidence interval: 75–100%) and even a 2-dose schedule (96%, 95% confidence interval: 88–99%) during a period of vaccine shortage [3]. Increasingly crowded immunization programs have prompted exploration of PCV-7 schedules with fewer doses and at present over half of the European countries have already implemented a 2 + 1-dose schedule, also to allow for programmatic differences and to reduce costs [4–6].

For non-inferiority comparison between pneumococcal conjugate vaccines, an individual anticapsular serum IgG antibody concentration of $0.35 \mu\text{g/ml}$ 1 month after the primary series in infants was estimated to be associated with clinical efficacy against IPD, at least in industrialized countries like the USA [7]. In non-western countries and high-risk populations this threshold may be higher and more around $1.0 \mu\text{g/ml}$ [8]. However, threshold protective antibody levels are not well understood and seem to differ per serotype. The levels needed to prevent carriage are higher and were suggested to be around $5.0 \mu\text{g/ml}$ which is considerably higher than what is thought to be required for prevention of invasive disease [2,8,9]. Higher levels may also be required for pneumonia and otitis media compared with IPD [10,11].

A non-randomized immunogenicity study in the UK comparing a 2 + 1- and 3 + 1-dose schedule showed no consistent distinct differences between both vaccine schedules as measured by geo-

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metric mean concentrations (GMC) per vaccine serotype [12]. In a recent immunogenicity study in Iceland, post-primary differences in IgG GMCs were found for several serotypes following 2 or 3 primary vaccinations, yet after the booster dose the only difference observed was for serotype 18C [13]. However, since both the UK and Iceland study were performed with experimental 9-valent CRM197-conjugated pneumococcal vaccines that may have different immunogenic capacity compared with the currently licensed PCV-7, potential differences between a 2 + 1- and 3 + 1-dose schedule may have been masked [12,13]. In 2005, in a single cohort of infants receiving a 2 + 1 PCV-7 schedule, Kaythy et al. demonstrated low pre-booster levels for serotypes 6B and 23F [14]. Following the booster vaccination however, no differences were seen.

Since immunogenicity studies comparing 2- and 3-dose primary schedules with the licensed 7-valent CRM197 pneumococcal conjugate vaccine in infants are scarce, we evaluated individual serotype responses with the currently used PCV-7 in a 2 + 1- and 3 + 1-dose schedule in infants with primary vaccination at 2 and 4 months or 2, 3 and 4 months of age and a booster dose at 11 months of age.

2. Subjects and methods

2.1. Study design

For this study we derived data from two separate cohorts in the Netherlands. The first study was a randomized controlled trial investigating the effects of reduced-dose PCV-7 schedules on pneumococcal carriage in the first 2 years of life (ISRCTN25571720) [15]. The participants were born between June and December 2005, 1 year before nationwide implementation of PCV-7 in June 2006. Infants younger than 12 weeks, not yet immunized and living in the study region were eligible for inclusion. Exclusion criteria were known immunodeficiency, craniofacial or chromosomal abnormalities, language barrier or expected relocation within the follow-up period [15]. Infants were randomized to receive 2 primary doses of PCV-7 at 2 and 4 months of age, followed by a booster dose at 11 months of age or no PCV-7 vaccinations (controls). Infants were included in the immunogenicity arm of the study on voluntary basis with blood sampling immediately before nasopharyngeal swabs were taken. Blood samples from infants receiving 2 primary doses without a booster dose of PCV-7 were collected at 12 months of age and included in the current analysis as pre-booster samples. Blood samples from infants receiving 2 + 1 doses were also collected at 12 months of age, 1 month after the booster dose at 11 months and included in the analysis as post-booster samples. No baseline differences were found in children who participated in the immunogenicity subset and infants participating in the main carriage trial.

The second group of infants participated in a serological immune-surveillance study on pertussis vaccination (ISRCTN97785537). The infants received a 3 + 1-dose PCV-7 schedule at the age of 2, 3, 4 and 11 months, according the Dutch national immunization program (NIP) which was implemented for all newborns from April 2006, without a catch-up program for older children [16]. Infants in good general health eligible for the fourth DTP-IPV-Hib vaccination were qualified for inclusion. Exclusion criteria were known immunodeficiency, a history of any neurologic disorder (including epilepsy) or previous vaccination with any other vaccine than those used in the NIP. We obtained blood samples at 11 months (included as pre-booster samples in the current analysis) and 1 month after the booster dose at age 12 months (included as post-booster samples in the current analysis) from infants born from April to July 2006. Inclusion was restricted to infants born within the first 3 months after PCV-7 introduction in the NIP.

From both studies blood samples from high-risk infants for hepatitis B that had concomitantly received Hepatitis B immunizations were excluded from analysis. Post-booster blood samples obtained outside the estimated range of 21–42 days after receiving the booster dose were excluded. For both schedules comparable percentages of blood samples were eligible for analyses.

2.2. Study vaccines

In both studies the licensed 7-valent CRM197-conjugated pneumococcal vaccine (Wyeth Pharmaceuticals) was administered, concomitantly with DTP-IPV-Hib immunizations. Since the vaccines of the 3 + 1-dose schedule were administered as part of the Dutch NIP, different lot numbers were used in the two studies. Of note is that in January 2006, the DTaP-IPV-Hib vaccine (Infanrix-IPV-Hib™, GlaxoSmithKline) in the Dutch NIP was replaced by a comparable DTaP-IPV-Hib vaccine containing additional *B. pertussis* proteins (Pediace™, Sanofi Pasteur MSD) [17]. Therefore, priming DTaP-IPV-Hib vaccinations differed between both study cohorts. Both cohorts received Pediace™ as a booster dose.

Informed consent was obtained from the parents or guardians of all study participants. Studies were approved by a national ethics committee.

2.3. Laboratory measurements

After collection blood was stored at 4 °C. Serum was separated within 24 h and stored at –20 °C until assayed. Serum IgG antibody levels were measured to the 7 vaccine pneumococcal polysaccharides 4, 6B, 9V, 14, 18C, 19F and 23F. All sera were assayed in the laboratory for infectious diseases of the National Institute for Public Health and the Environment in Bilthoven with ELISA using double adsorption with cell wall polysaccharide and 22F polysaccharide [18].

2.4. Statistical analysis

Results of IgG antibody levels are expressed in Geometric Mean Concentration (GMC) with 95% confidence interval (95% CI). Statistical differences in IgG GMC values were assessed by log transformed unpaired *t*-test. Differences in percentages of subjects with antibody levels ≥ 0.35 µg/ml, ≥ 1.0 µg/ml and ≥ 5.0 µg/ml were calculated using Fisher's exact test. All reported *p*-values are 2-sided, *p*-values < 0.05 were considered significant. The study sample sizes enabled an estimation of pneumococcal GMCs with 95% CI within 1.4-fold and detection of a 2-fold difference for comparing schedules with 80% power at a 5% significance level [12]. Analyses were performed with SPSS 15.0.

3. Results

3.1. Study participants

We collected 80 pre-booster and 72 post-booster serum samples from infants receiving the 2 + 1-dose schedule and 98 pre-booster and 90 post-booster serum samples from infants receiving the 3 + 1-dose schedule.

For the pre-booster serum samples baseline characteristics of the participants (gender, age at time of blood collection) were comparable between the two vaccination schedules. For the post-booster samples, infants receiving the 3 + 1-dose schedule were up to 1 month older at the time of the booster vaccination compared to the infants receiving the 2 + 1-dose schedule (mean age 12.1 months vs. 11.3 months; *p* < 0.001).

Table 1

Pre- and post-booster serotype specific IgG antibody levels (GMC) in infants receiving a 2 + 1 and 3 + 1 PCV-7 schedule with early primary vaccinations at 2, 3 and 4 months or 2 and 4 months of age.

Serotype	Pre-booster samples; GMC ($\mu\text{g/ml}$) (95% CI)			Post-booster samples; GMC ($\mu\text{g/ml}$) (95% CI)		
	2 primary dose ($n = 80$)	3 primary dose ($n = 98$)	p -Value ^a	2 + 1-dose ($n = 72$)	3 + 1-dose ($n = 90$)	p -Value ^b
4	0.28 (0.23–0.34)	0.30 (0.26–0.34)	NS	2.66 (2.26–3.12)	3.05 (2.57–3.61)	NS
6B	0.23 (0.19–0.30)	0.40 (0.31–0.49)	0.002 [#]	2.26 (1.64–3.12)	4.73 (3.62–6.17)	0.001 [#]
9V	0.27 (0.22–0.33)	0.31 (0.27–0.36)	NS	2.21 (1.90–2.58)	2.36 (2.00–2.78)	NS
14	1.76 (1.39–2.24)	1.56 (1.23–1.81)	NS	9.43 (7.76–11.46)	10.32 (8.62–12.34)	NS
18C	0.19 (0.16–0.23)	0.22 (0.18–0.25)	NS	1.97 (1.66–2.34)	1.91 (1.61–2.27)	NS
19F	0.94 (0.76–1.16)	0.96 (0.74–1.24)	NS	3.43 (2.85–4.12)	4.80 (4.01–5.76)	0.012 [#]
23F	0.21 (0.16–0.27)	0.22 (0.18–0.26)	NS	2.61 (2.12–3.21)	3.15 (2.54–3.92)	NS

NS, non-significant.

^a p -Values of 2 primary dose vs. 3 primary dose. Calculated using log transformed unpaired t -test.

^b p -Values of 2 + 1-dose vs. 3 + 1-dose. Calculated using log transformed unpaired t -test.

[#] p -Value < 0.05.

3.2. Pre-booster antibody levels

No differences in pre-booster IgG GMCs between the 2 schedules for 6 out of 7 PCV-7 serotypes were observed. Pre-booster GMCs ranged per serotype from 0.19 $\mu\text{g/ml}$ for 18C to 1.76 $\mu\text{g/ml}$ for serotype 14 (Table 1). The single exception was serotype 6B for which lower pre-booster GMCs were observed (0.23 $\mu\text{g/ml}$ vs. 0.40 $\mu\text{g/ml}$, $p = 0.002$) after 2 and 3 primary doses, respectively. Both vaccination schedules did not differ in the proportion of infants with pre-booster antibody levels $\geq 0.35 \mu\text{g/ml}$, $\geq 1.0 \mu\text{g/ml}$ or $\geq 5.0 \mu\text{g/ml}$, with the exception of 6B. For serotype 6B, 26% of infants who received a 2 + 1-dose schedule showed antibody levels of $\geq 0.35 \mu\text{g/ml}$ compared with 52% in the 3 + 1-dose group ($p = 0.001$) (Fig. 1).

3.3. Post-booster antibody levels

One month after the booster dose, IgG GMCs were similar for the 2 + 1- and 3 + 1-dose schedule, except for 6B and 19F (Table 1), where the GMCs for serotype 6B were 2.26 $\mu\text{g/ml}$ vs. 4.73 $\mu\text{g/ml}$ ($p = 0.001$) and for 19F 3.43 $\mu\text{g/ml}$ vs. 4.80 $\mu\text{g/ml}$ ($p = 0.012$), respectively. Overall, the lowest GMCs were seen for serotype 18C. Comparing the proportion of children reaching threshold antibody concentrations, the percentages of infants with antibody levels $\geq 0.35 \mu\text{g/ml}$ were comparable between both vaccination schedules for all 7 serotypes (Fig. 1). The percentages of infants reaching antibody levels $\geq 1.0 \mu\text{g/ml}$ differed between the two vaccination schedules for serotype 6B, with 73.6% vs. 88.9% of the infants reaching $\geq 1.0 \mu\text{g/ml}$ after the 2 + 1-dose vs. the 3 + 1-dose schedules, respectively ($p = 0.014$). Differences were also observed for the percentages of infants with antibody levels $\geq 5.0 \mu\text{g/ml}$ for serotypes 6B and 19F; 27.8% vs. 52.2% ($p = 0.002$) for 6B and 27.4% vs. 44.4% ($p = 0.034$) for 19F after a 2 + 1- or 3 + 1-dose schedule, respectively. For serotype 23F 19.4% vs. 33.3% of the infants reached antibody levels $\geq 5.0 \mu\text{g/ml}$ ($p = 0.053$).

4. Discussion

This study compared the pre- and post-booster IgG antibody levels for the 7 vaccine serotypes following a 2 + 1- and a 3 + 1-dose schedule with the currently used 7-valent pneumococcal conjugate vaccine with early primary doses between 2 and 4 months of age. Post-primary antibody levels $\geq 0.35 \mu\text{g/ml}$ were estimated to be an indication for protection against IPD [7]. No pre- and post-booster threshold values have been defined around 1 year of age, but a post-booster value of 1.0 $\mu\text{g/ml}$ may give some indication of protection around the age of the booster vaccination or for more vulnerable populations [8]. This study showed that pre- and post-booster antibody levels were comparable between a 2 + 1- and 3 + 1-dose PCV-7

schedule, with similar proportions of infants that reached post-booster antibody levels above $\geq 0.35 \mu\text{g/ml}$ and $\geq 1.0 \mu\text{g/ml}$ for 5 of the 7 serotypes. Exceptions however were serotype 6B and 19F. Between the two vaccination schedules a difference of >10% was observed in pre- and post-booster GMCs for serotype 6B and in the post-booster GMCs for serotype 19F. These results with the licensed PCV-7 differ somewhat from the results with the experimental 9-valent vaccines in the UK and Iceland, where heights of the antibody levels following the booster dose after 2- and 3-dose primary schedules were comparable for 6B and 19F [12,13]. This may be due to a different immunogenicity of the experimental 9-valent pneumococcal conjugate vaccines used in these studies. However, the age of the infants at the primary injections and timing of the booster vaccination as well as other factors like concomitant childhood vaccinations or ethnic background variability may have impact on immunogenicity of vaccines [19,20]. Difference in natural boosting of the immune system in the other countries by circulating vaccine strains may have affected the height and persistence of antibody levels and booster responses. On the other hand, the current 2 + 1-dose study as well as the UK immunogenicity study were both performed well before widespread PCV-7 implementation in the NIP and pneumococcal carriage levels and individual circulating serotypes were comparable between the Netherlands and the United Kingdom [15,21]. Also antibody concentrations from the 3 + 1-dose schedule in children born during the first 3 months of implementation of PCV-7 in the NIP are unlikely to be much affected by diminished natural boosting, since no catch-up was done in the Netherlands [16]. Very few children under 5 years of age had been immunized with PCV-7 before national implementation of PCV-7 and no herd effects were observed for IPD within the first 2 years after June 2006 (Rodenburg et al., submitted). We found no differences in pre- and post-booster GMCs for serotype 18C, unlike the Icelandic study where lower post-booster levels were observed after reduced schedules with a CRM197-conjugated 9-valent pneumococcal-meningococcal C vaccine [13]. However, identical to the Iceland study, lowest antibody levels were observed for serotype 18C. In our study the primary series were administered at 2, 3, 4 or 2 and 4 months of age. It is possible that the extra dose at 3 months of age did not add much to the overall induction of IgG antibodies because of the relatively young age and the short 1-month interval between doses. A schedule given in the United States with 3 primary doses at 2, 4 and 6 months of age is potentially more immunogenic than 2 primary doses at 2 and 4 months of age [19]. Unfortunately, we do not have data on post-primary responses or data on comparing time intervals between schedules. For meningococcal conjugate vaccines earlier research showed higher booster responses after less primary dose, however this effect was not seen for the 7-valent pneumococcal conjugate vaccine [22].

Fig. 1. Reverse cumulative distribution curves of infants receiving a 2 + 1 and 3 + 1 PCV-7 schedule with early primary vaccinations at 2, 3 and 4 months or 2 and 4 months of age. Vertical lines indicate levels above 0.35 $\mu\text{g/ml}$, 1.0 $\mu\text{g/ml}$ and 5.0 $\mu\text{g/ml}$.

Successful immunological priming is important for protection in particular in the first year of life during the peak incidence of IPD between the primary series and the booster dose [1]. Looking at the results from Iceland, post-primary GMC differences were found between the 2 primary dose vs. 3 primary dose schedules, which were no longer existent in the pre-booster antibody levels at 12 months of age [13]. In contrast, these differences were not seen in post-primary antibody levels from the United Kingdom between the 2 primary dose vs. 3 primary dose schedules [12]. We found differences for 6B and 19F and possibly for 23F. Protective levels seem to vary between serotypes and lower antibody levels may suffice for clinical protection as was shown for serotype 6B IPD [2]. Furthermore, in our study serotype 6B antibody levels were in a similar range of most other vaccine serotypes and well above serotype 18C that showed the lowest pre- and post-booster antibody lev-

els in both our vaccination groups. The question is whether the dose-dependent antibody responses with the currently used PCV-7 may have clinical consequences. Looking at other clinical data, a surveillance report from Norway on the first 2 years after national implementation of a 2 + 1-dose schedule (3, 5 and 12 months with catch up for infants aged 3–6 months) showed a strong decline in IPD for all vaccine serotypes including the serotypes 6B, 18C, 19F and 23F with no vaccine failures [23]. However, the later primary schedule at 3 and 5 months and the addition of a catch-up program for infants aged 3–6 months might have masked lower protection in vaccinated children after reduced primary doses since herd effects may have attributed to effectiveness due to reduction of circulating vaccine strains in the population.

Effects of reduced-dose schedules on disease in particular respiratory pneumococcal infections like pneumonia and otitis media

also need to be evaluated since higher antibody levels may be required for protection. For protection against nasopharyngeal acquisition serum antibody levels above 5.0 µg/ml have been suggested [9]. We found differences in proportions of children reaching antibody above 5.0 µg/ml for serotypes 6B, 19F and possibly 23F. For serotype 23F, the difference in the proportion of children reaching the 5 µg/ml level was not significantly different ($p=0.053$), but this is likely due to the sample size of the children. Previously Jokinen et al. found a GMC of 0.5 µg/ml to be more than 65% efficacious against for serotype 6B otitis media, but for serotype 19F this level had negligible protection [10]. We recently reported in our carriage study that although serotype 19F showed a decline at 18 months after 2 + 1 doses compared with unvaccinated controls, this was no longer present at 24 months of age. In contrast, serotypes 6B and 23F showed around 80% decline in carriage compared to unvaccinated controls at 24 months of age after a 2 + 1-dose schedule [15]. This highlights the serotype dependent efficacy of antibody concentrations or of other antibody characteristics like affinity [10]. From Italy and Quebec first reports show a decline in respiratory disease like otitis media and pneumonia after national PCV-7 implementation in reduced-dose 2 + 1 schedules, but again in these countries catch-up programmes might contribute to this observed decrease by herd effects [24,25]. Moreover, these surveillance data cannot discriminate between serotype specific protection against respiratory disease. Differences found for serotype 6B, 19F and 23F can potentially impair the effectiveness of 2 + 1-dose schedules for prevention of respiratory disease and also herd effects in particular before 2 years of age [26,27]. However, based on our carriage study, we predict that indirect protection via herd effects for all serotypes will eventually be obtained for both invasive and respiratory disease after a 2 + 1-dose schedule with primary doses also at 2 and 4 months of age followed by a booster at 11 months of age, since in the second year of life at 18 and 24 months a significant overall 60% reduction of vaccine serotypes was observed [15]. Noteworthy, our carriage study also showed that the age of carriage reduction of serotypes 6B and 19F may be dose dependent before 24 months of age with earlier reduction after more doses of PCV-7 [15].

Our study has several limitations. Since the two schedules were performed in a non-randomized setting in two different cohorts some potential confounders should be assessed. Firstly, the co-administration of different DTaP-IPV-Hib vaccines in the study groups may have affected results. However, a study assessing the compatibility of concurrently administered PCV-7 with acellular pertussis vaccine showed no differences in immune responses to PCV-7 between groups receiving the vaccines concomitantly or separately [28]. Secondly, different lot numbers of PCV-7 were used in the two studies. Thirdly, there was difference in the age of the children receiving the booster dose between study groups. Infants receiving the 3 + 1-dose schedule were up to 1 month older when receiving the booster compared with the infants receiving the 2 + 1-dose schedule. However, when comparing only infants who received their booster dose before the age of 12 months in both schedules, the difference for 6B persisted but no significant differences were observed for serotype 19F. However, the small group size does not allow firm conclusions (see also [supplementary table](#)). Furthermore, all other inclusion- and exclusion criteria as well as baseline characteristics between the two vaccination schedules were comparable. Fourth, the different time periods in our study may have introduced herd effects and less natural boosting by the vaccine serotypes for the 3 + 1-dose group. However, our carriage study that followed children up till their second birthday during the period June 2005 till February 2008, and thus including the study period of both the 2 + 1- and 3 + 1-dose groups, no signs of herd effects were observed in unvaccinated controls [15]. Lastly, no data about post-primary IgG antibody levels were available so

the period between primary vaccinations and booster vaccination cannot be compared.

To summarize, we found comparable pre- and post-booster antibody responses for most vaccine serotypes between a 2 + 1- and 3 + 1-dose vaccination schedule with the currently used 7-valent pneumococcal conjugate vaccine. Between the two vaccination schedules a difference was observed in pre- and post-booster antibody levels for serotype 6B and in the post-booster antibody levels for serotype 19F. However, first surveillance reports seem to indicate clinical protection against invasive disease also with reduced-dose schedules. A recent carriage study indicated that herd effects due to diminished circulation of vaccine serotypes are to be expected after 2 + 1-dose schedules that will decrease also vaccine serotype respiratory disease like otitis media and pneumonia.

Conflict of interest

Supported by the Dutch Ministry of Public Health, Welfare and Sports/Netherlands Vaccine Institute. Dr. Sanders reports receiving unrestricted grants from Wyeth and Baxter for research, consulting fees for Wyeth and GlaxoSmithKline, lecture fees from Wyeth and grant support from Wyeth and GlaxoSmithKline for vaccine studies. Dr. Veenhoven received research grants for pneumococcal vaccine studies from GSK. For all other authors no potential conflicts reported.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.vaccine.2009.10.151](https://doi.org/10.1016/j.vaccine.2009.10.151).

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